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EUROPEAN CYTOGENETICISTS ASSOCIATION

E.C.A. NEWS LETTER

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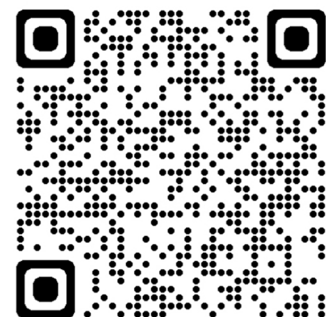

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E.C.A. Newsletter

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Editor of the E.C.A. Newsletter:

Konstantin MILLER
Institute of Human Genetics
Hannover Medical School, Hannover, D
E-mail: miller.konstantin@mh-hannover.de

Editorial committee:

J.S. (Pat) HESLOP-HARRISON
Genetics and Genome Biology
University of Leicester, UK
E-mail: phh4@le.ac.uk

Kamlesh MADAN
Dept. of Clinical Genetics
Leiden Univ. Medical Center, Leiden, NL
E-mail: k.madan@lumc.nl

Mariano ROCCHI
President of the E.C.A.
Dip. di Biologia, Campus Universitario
Bari, I
E-mail: mariano.rocchi@uniba.it

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E.C.A. on Facebook

As mentioned in earlier Newsletters, E.C.A. is on Facebook.
You will find announcements of interesting articles, related to cytogenomics or to biology in general, and also pictures and stories from social events related to E.C.A. and its members. Also our E.C.A. conferences will be covered on Social Media.
You can see the weekly posts and announcements via the direct link

https://www.facebook.com/Cytogenetic/ or on the updated E.C.A. website **http://www.e-c-a.eu/**

You will find a selection of interesting Facebook posts in this Newsletter starting at page 84.
Please contact us (mariano.rocchi@uniba.it) if you wish to share an interesting news item or a pertinent article.

President's Address

Dear ECA members, dear friends,

The ECA conference is held every two years. This year the conference will be in Montpellier 1 – 4 July 2023. The last onsite conference was in Salzburg, in 2019, four years ago. The 2021 conference was held online due to Covid-19. Thus, it is after four years that we will have the opportunity to see each other again, as a community, which started with chromosomes but which has continued to widen its horizons. The conference is now called the European Cytogenomics Conference.

Two courses complement the educational mission of the conferences. The well-established course on Classical and Molecular Cytogenetics is held each year in Nîmes. The next Nîmes course will be in March 2024. The course on Clinical Cytogenetics, that is held in Goldrain Castle, North Italy, was started 16 years ago by Prof. Albert Schinzel, director of the course. It has always been supported by the ECA but now ECA is much more involved in the organization to help continue this much appreciated course. This year the Goldrain course will take place from 21 to 29 August. At the moment there are still some places available...
You will find information about both courses on these web pages:

http://www.biologia.uniba.it/SEC/
or
https://www.e-c-a.eu/EN/ECA-Courses.html

The ECA conferences and courses are excellent opportunities to update our knowledge in the various fields of cytogenomics, to learn about new technological approaches and, most importantly, to meet and exchange experiences and knowledge.
This very thought was expressed in an email I received from the head of a laboratory, who introduced two of his students to a course, saying "I myself am a graduate of the ECA cytogenetics course in Nimes of the 2016 and I came back with a lot of knowledge".

Montpellier is also known as the city of medicine, which has the oldest practicing medical school in the world and is at the forefront of various scientific fields. It is also a city of art and history, and, of course, gastronomy! All this promises to make our stay in Montpellier unforgettable.

In the present situation members from some countries may not be able to attend the conference. We sincerely hope that the next conference in 2025 will be held in better and peaceful circumstances.

Looking forward to seeing you in Montpeller,

Kind regards,

Mariano Rocchi
E.C.A. President

14th European Cytogenomics Conference Montpellier - Program

Saturday 1 July

14:30-17:30 **Workshops and discussion meetings of Permanent Working Groups**

14:30-16:30 **PWG Quality Issues, Training and Cytogenomics and ISCN Workshop, Room Barthez, Coordinators: Martine Doco-Fenzy, Jean-Michel Dupont**

14:30	**Martine Doco-Fenzy**: Introduction and news about the update of ISO 15189
14:45	**Melody Tabiner:** External quality assessments, contribution to quality improvement
15:15	**Ros Hastings**: Genome Mapping (optical and electronic) nomenclature and ISCN 2024 (P1003)
15:45	**Jean-Michel Dupont**: Training on ISCN: Quiz
16:15	**Marie Bérengère Troadec**: Towards a decision-making tool for the identification of chromosome structural abnormalities in conventional cytogenetics: Development of a prototype for the detection of del(5q) deletion based on artificial intelligence (P1053)
16:30	**Closing**

14:30-16:30 **PWG Neoplasia, Room Sully 2, Coordinators: Paola Caria, Harald Rieder, Roberta Vanni**

14:35	**Francesco Pasquali**: Donor cell acute myeloid leukaemia after haematopoietic stem cell transplantation for chronic granulomatous disease (P1067)
14:48	**Kalliopi Manola:** Ring chromosomes in hematological malignancies are mainly associated with myeloid malignancies and complex karyotypes (P1023)
15:01	**Uliana Karnaukhova:** Cytogenetic groups of pediatric acute myeloid leukemia from Ukraine (P1120)
15:14	**Victoria Marcu**: Validation of the OGM for cytogenomic testing in hemato-oncology – Sheba Medical Center experience (P1052)
15:27	**Bob Argiropoulos:** Laboratory Validation and Clinical Implementation of an RNA sequencing-Based Prognostic Assay for Multiple Myeloma (P1155)
15:40	**Break**
15:50	**Gulsim Smagulova:** Mutation of the PIK3CA gene in breast cancer (P1069)
16:03	**Halka Lhotska**: Detection of promoter methylation as well as deletion of MGMT gene in patients with glioblastoma using methodologically different approaches (P1097)
16:16	**Tadeusz Kałużewski**: Report on the implementation of an early cancer identification and prevention program among the population of central Poland (P1128)
16:30	**Closing**

14:30-15:30 **PWG Prenatal Diagnosis, Room Sully 3**
Coordinators: Rosário Carvalho Pinto Leite, Jean-Michel Dupont

14:30	**Jean-Michel Dupont**: Presentation of the guidelines of Microarrays in Prenatal Diagnosis
14:40	**Rosário Carvalho Pinto Leite**: Presentation of the results of the survey
14:50	**Celine Dupont**: The ever-changing face of Cytogenetics Units: Use and contribution of Whole Exome Sequencing in prenatal diagnosis (P1029)
15:05	**Joris Vermeesch**: A larger European network about cancers in pregnancy
15:30	**Closing**

15:30-17:30 **PWG Clinical and Molecular Approaches to Cytogenetic Syndromes & Cytogenomics, Room Rondollet, Coordinators: Anna Lindstrand, Damien Sanlaville, Joris Vermeesch**

15:30	**Vasheghani Farahani Faezeh**: Optical Genome Mapping: Comparing OGM with other Cytogenomics technologies. Experience on 60 individuals with developmental or fertility disorders (P1032)
15:42	**Anna Lokchine**: Primary Ovarian Insufficiency: don't neglect intragenic CNVs (P1062)
15:54	**Vladimíra Vallová**: Different strategies for the detection of copy-number variations from exome sequencing data (P1037)

16:06	**Vincent Gatinois**: Breastfeeding promotes persistence of the mother's chimeric cells in their offspring (P1157)
16:18	**Nicolas Chatron**: Streamlining cytogenetics analysis of genome sequencing data: a comprehensive guide for Balanced Structural Variants (P1139)
16:30	**Annelies Dheedene**: Copy number detection in exome sequencing data for patients with neurodevelopmental disorders: an effective approach (P1154)
16:42	**Leona Morožin Pohovski**: First case report of a patient with three copies of distal 16p12.1p11.2 (BP1-BP3 region) and four copies of proximal 16p11.2 (BP4-BP5 region) inherited from both parents (P1154)
17:04	**Anna Lengyel**: Expanding the phenotype of 14q11.2 microdeletions encompassing CHD8 and SUPT16H genes (P1065)
17:30	**Closing**
15:30-17:30	**PWG Animal, Plant, and Comparative Cytogenetics, Room Sully 3** **Coordinators; Pat Heslop-Harrison, Trude Schwarzacher**
15:30	**Pat Heslop-Harrison**: Introduction
15:40	**Magdalena Chmielewska**: Gametogenesis in hybridogenetic frogs – tracking cellular events of genome elimination and endoreduplication (P1012)
15:55	**Anna Dudzik**: Cytogenetics of the hybrid frog Pelophylax grafi and its parental species Pelophylax perezi (P1021)
16:10	**Francesca Dumas**: CAP-A satellite DNAs probe mapping on Sapajus cay paraguay and S. macrocephalus by FISH (Platyrrhini, Primates) (P1035)
16:25	**Trude Schwarzacher**: Chromosomal evolution and genome expansion in diploid oats
16:40	**Ioana Nicolae**: Cytogenetic screening of Romanian bovine breeds (P1098)
16:55	**Verónica Mestre**: A glimpse of the karyotype reshuffling from human to Myotis blythii (Vespertillionidae, Chiroptera) (P1125)
17:10	**Eleonora Pustovalova** Evolution of gametogenic pathways in reproduction of hybrid males from Pelophylax esculentus complex (P1044)
17:25	Summary/general discussion
17:30	**Closing**
16:30-17:30	**PWG Chromosomes' Integrity, Stability and Dynamics, Room Barthez** **Coordinators: Jose Garcia-Sagredo, Emanuela Volpi**
16:40	**Eliane El Achkar**: Molecular Mapping of Two Replication Stress-Induced Hotspots of Breakage at the Common Fragile Site FRA11D Harboring Cancer and Neurological Genes (P1068)
16:48	**Mateus de Oliveira Lisboa**: Chromosomal Instability in Mesenchymal Stromal Cells From Acute Myeloid Leukemia Patients (P1057)
17:56	**Anna Schachner**: Monitoring of long-term cultured induced pluripotent stem cells by Optical Genome Mapping (OGM) confirms sustained fine-structural genomic stability across more than 60 in vitro passages (P1101)
17:04	**Nicoletta Selenti**: Cytogenetic analysis of induced pluripotent stem cell (iPSC) cultures derived from dermal fibroblasts (1046)
17:12	**Radhia M'Kacher**: Screening of biomarkers for chromosomal instability in the cytogenetic clinic: Present status on technological advances and their implementation into routine screening programs (P1151)
17:30	**Closing**
16:30-17:30	**PWG Marker Chromosomes, Room Sully 2** **Coordinators: Thomas Liehr, Isabel Marques-Carreira**
16:30	**Thomas Liehr**: Introdution on Small Supernumerary Marker Chromosomes (sSMC)
16:40	**Christina Pérez**: Optical Genome Mapping (OGM): Validation and characterization of marker chromosomes (P1095)
16:50	**Joana Melot**: Importance of arrayCGH for sSMC detection and characterization
17:00	**Esther Cuatrecasas**: Classical genetic techniques are still in use: a case with low mosaicism (P1010)
17:10	**Thomas Liehr**: Most complex sSMC ever, as yet!
17:30	**Closing**

Saturday 1 July

18:00-19:00	Conference Opening lecture **Chair: Mariano Rocchi and Franck Pellestor**
	Eva R. Hoffmann: Aneuploidy in the Maternal Germline

Sunday 2 July

8:30-10:15	Plenary session 1 **- Mosaicism: from Preimplantation Embryos to Aging** **Chairs: Joris Vermeesch and Elisabeth Syk Lundberg**
8:30-9:00	**Antonio Capalbo**: Mosaicism in Preimplantation Embryos
9:00-9:30	**Malgorzata I. Srebniak**: Mosaicism in Prenatal Diagnosis: from NIPT to Amniocytes Investigation
9:30-10:00	**Lars A. Forsberg**: Hematopietic Loss of Chromosome Y and Higher Mortality in Men
	Selected abstract
10:00-10:15	**Cornelia Daumer-Haas**: Normal Array-CGH Results in a Patient With Short Stature and Global Developmental Delay Carrying a de novo Ring Chromosome 2p and a Chromosome 2q Derivative With a Neocentromere
10:15-10:45	Coffee break
10:45-12:00	Plenary session 2 **- Cancer Cytogenomics** **Chairs: Felix Mitelman and Roberta Vanni**
10:45-11:15	**Sarah McClelland**: Replication Stress Generates Distinctive Landscapes of DNA Copy Number Alterations and Chromosome Scale Losses in Cancer
11:15-11:45	**Uri Ben-David**: Whole-Genome Duplication Shapes the Aneuploidy Landscape of Human Cancers
	Selected abstract
11:45-12:00	**Christina Srouji**: Optical Genome Mapping for Multiple Myeloma: Evaluation of The Technology in a Clinical Laboratory.
12:00-14:30	**Poster session and Satellite Symposia**
14:30-15:45	Concurrent Session 1 **- Recent Advances in Cytogenomics** **Chairs: Franck Pellestor and Harald Rieder**
14:30-15:00	**Alex Hoischen**: Optical Mapping to Karyotype
15:00-15:30	**Antonio Rausell**: Artificial Intelligence in Cytogenetics
	Selected abstract
15:30-15:45	**Gil Nifker**: Dam Assisted Fluorescent Tagging of Chromatin Accessibility (DAFCA) for Optical Genome Mapping in Nano-Channel Arrays
14:30-15:45	Concurrent Session 2 **- Beyond Genome Sequencing: the Epigenetic Signature** **Chairs: Orsetta Zuffardi and Joan Blanco**
14:30-15:00	**Bekim Sadikovic**: DNA Methylation Episignatures Associated with Large Structural Copy Number Variants: Clinical Implications
15:00-15:30	**Karen Temple**: Multi-locus imprinting disorders
	Selected abstract
15:30-15:45	**Mathilde Geysens**: Long Read Whole Genome Sequencing for The Detection of Structural and Epigenetic Variation in Developmental Disorders
15.45-16.15	Coffee break
16:15-17:30	Plenary session 3 **- Newly Emerged Technologies in Cytogenomics** **Chairs: Pat Heslop-Harrison and Emanuela Volpi**
16:15-16:45	**Brian Beliveau**: Paint-SHOP; Genome-Scale Oligonucleotide FISH Experiments
16:45-17:30	**Pat Heslop-Harrison and Emanuela Volpi**: Interactive Discussion; Massive Oligonucleotide Pools to Track Organization and Evolution of Chromosomes and Genomes
17:30-18:30	**Poster session**

Monday 3 July

8:30-10:30	Plenary session 4 - **Clinical Cytogenomics I** **Chairs: Damien Sanlaville and José Garcia-Sagredo**
8:30-9:00	**Anna Lindstrand**: Complex Genomic Rearrangements: an Underestimated Cause of Rare Diseases
9:00-9:30	**Orsetta Zuffardi**: Distal Germ-Line Deletions in Mosaic With Copy-Neutral Loss of Heterozygosity: Something to Be Considered in Genetic Counselling
9:30-10:00	**Brunella Franco**: From Gene Disruption to Missense Variants: how Different Types of Variants Influence the X-Linked Inheritance Model
	Selected abstracts
10:00-10:15	**Niels Tommerup**: Burden of Long Range Position Effects in Balanced Chromosomal Rearrangements
10:15-10:30	**Marlene Ek**: Multiomic Profiling Unravels Disease Mechanisms in Complex Chromosomal Rearrangements and Marker Chromosome Carriers
10:30-11:00	Coffee break
11:00-12:15	Concurrent Session 3 - **Clinical Cytogenomics II** **Chairs: Orsetta Zuffardi and Martine Doco-Fenzy**
11:00-11:30	**Nicolas Chatron**: Structural Variants in Clinical Practice Using Genome Sequencing
11:30-12:00	**Caroline Schluth-Bolard**: Constitutional Chromoanagenesis: From Diagnosis to Genetic Counselling
	Selected abstract
12:00-12:15	**Sanam Khan**: Systematic X-Inactivation Studies of Sequence Resolved Balanced X Chromosomal Rearrangements
11:00-12:15	Concurrent Session 4 - **Animal and Plant Cytogenomics I** **Chairs: Tony Heitkam and Trude Schwarzacher**
11:00-11:30	**Mathieu Rouard**: Comparative Genomics and Tools for Studying Chromosome Evolution
11:30-12:00	**Brankica Mravinac**: Coleopteran Satellite Profiles: Chromosomal and Sequence Organization
	Selected abstract
12:00-12:15	**Maria Filomena Lopes Adega**: A physical map of repetitive elements in the genomes of Iberian Peninsula chiropteran species
12:15-14:30	**Poster session and Satellite Symposia**
14:30 - 15:45	Plenary session 5**: Nuclear Organization and Diseases** **Chairs: Jean-Michel Dupont and Emanuela Volpi**
14:30 - 14:55	**Giacomo Cavalli**: The Role of 3D Genome Organization in The Regulation of Gene Expression and Cell Fate (to be comfrmed)
15:00-15:30	**Irina Solovei**: Spatial Organization of Transcribed Eukaryotic Genes
	Selected abstract
15:30-15:45	**Paola Caria**: 3D Nuclear Architecture Distinguishes Thyroid Cancer Histotypes
10:45-16:15	Coffee break
16:15-17:30	Concurrent Session 5 - **Animal and Plant Cytogenomics II** **Chairs: Trude Schwarzacher and Brankica Mravinac**
16:15-16:45	**Tony Heitkam**: Adding a Chromosome Perspective to Plant Genomics: Making Sense of Retained Retroviruses, Moving Retrotransposons and Expanding Satellite DNAs

16:45-17:15	**Yi Tzu Kuo**: Plasticity in centromere organization: A few megabased-sized centromere units can form a holocentromere
	Selected abstract
17:15-17:30	**Alla Krasikova**: The first nuclear and cytoplasmic whole transcriptome profile of chicken oocytes at the lampbrush chromosome stage
16:15-17:30	Concurrent Session 6 **- Accreditation, Quality Control and Education** **Chairs: Konstantin Miller and Martine Doco**
16:15-16:45	**Folker Spitzenberger**: The New ISO 15189 Standard Medical Laboratories
16:45-17:15	**Johan den Dunnen**: Sequence-based Nomenclature and the Novelties to Come in the Next ISCN Version
	Selected abstract
17:15-17:30	**Mathilde Quibeuf**: Educational Benefits of Analysing Highly Complex Chromosomal Rearrangements Such as Chromoanagenesis by Long Read Approaches
17:30-18:30	Poster session

Tuesday 4 July

8:30-10:30	Plenary session 6 **- Prenatal Diagnosis and Preimplantation** **Chairs: Jean-Michel Dupont and Rosário Pinto Leite**
8:30-9:00	**Robert-Jan H. Galjaard**: Genome-Wide Noninvasive Prenatal Testing: Follow-Up Results of the TRIDENT-2 Study
9:00-9:30	**Joris Vermeesch**: Fragmentomics and Non Invasive Prenatal Screening (NIPS)
9:30-10:00	**Lyn Chitty**: Prenatal Diagnostic Yield and Pitfalls Through Arrays, Exome, and NIPT
	Selected abstracts
10:00-10.15	**Armelle Duquenne**: Multicentric Longitudinal Performance Monitoring of Different non-Invasive Prenatal Screening Technologies Used in Belgium
10:15-10.30	**Ludovica Picchetta**: Triploid Conceptions Are Predominantly Caused by Female Meiosis II Errors and Their Risk Increases with Advancing Maternal Age
10:30-10.50	Coffee break
10:50-11.30	Satellite Symposia
11:30-12:20	**Closing keynote** **Chairs: Mariano Rocchi and Thierry Lavabre-Bertrand** **Michael E.Talkowski**: The Landscape of Structural Variation Across Diverse Global Populations and Developmental Disorders
12:20	**Closing ceremony**

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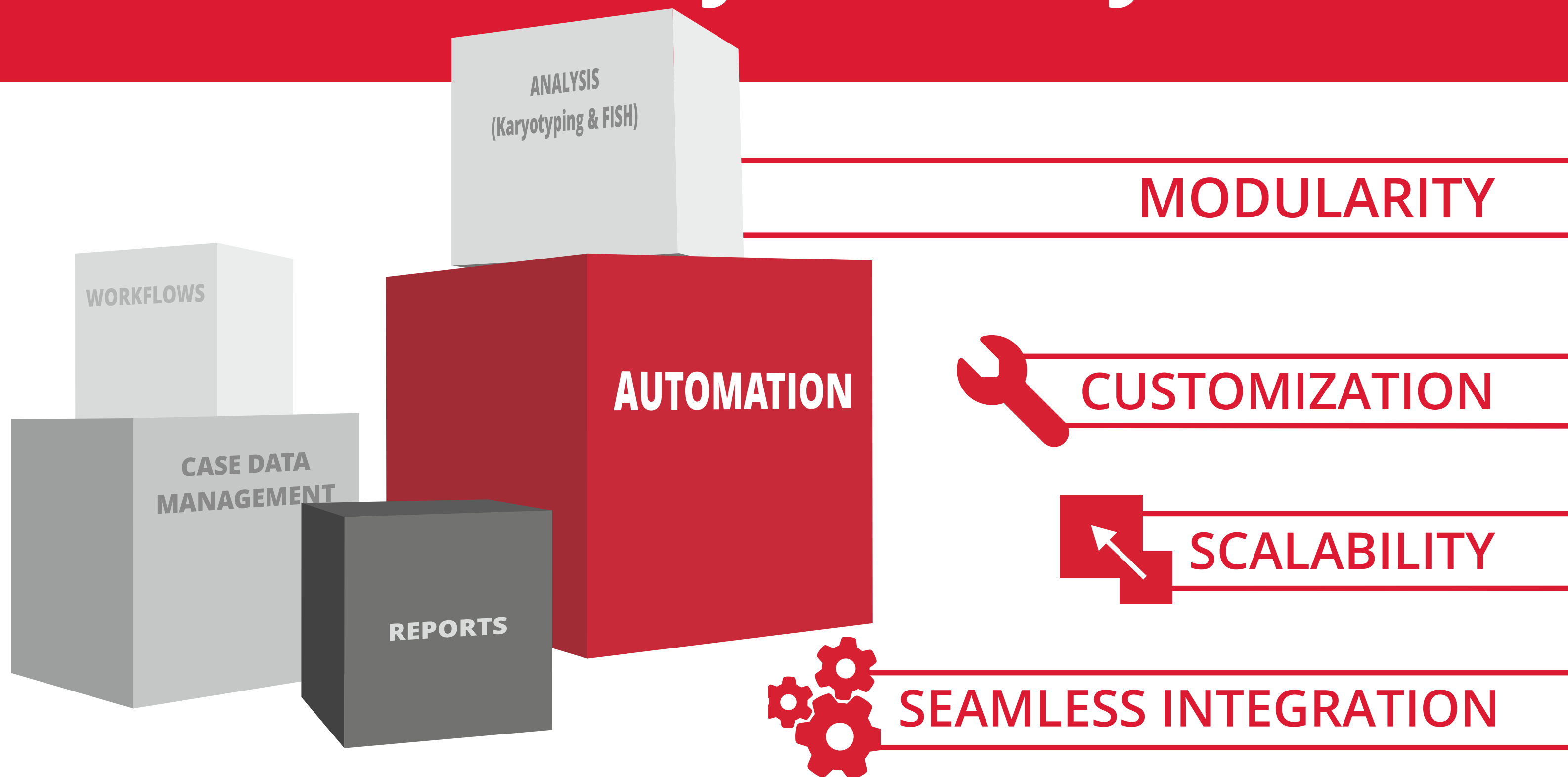

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Abstracts - Invited Lectures

L1 Aneuploidy in the Maternal Germline

Eva R. Hoffmann

L2 Mosaicism in Preimplantation Embryos

Antonio Capalbo
antcapalbo@gmail.com
Juno Genetics, Reproductive genetics

We have known for a long time that human embryos are frequently affected by whole-chromosome gains and losses (aneuploidies). The vast majority of embryonic aneuploidies are caused by maternal meiosis errors, which result in aneuploid eggs. After fertilization, such altered oocytes are likely to pass on their chromosomal defects to every embryonic cell. These aneuploidies are commonly referred to as uniform aneuploidies of meiotic origin. Preimplantation genetic testing for aneuploidies (PGT-A) is a test that allows the embryonic chromosomal status to be investigated prior to transfer. PGT-A has been shown to be a reliable method for detecting uniform aneuploidies in preimplantation embryos. It is highly reproducible and accurate, with high clinical predictive value for a normal or uniformly aneuploid diagnosis. It has been demonstrated that uniformly aneuploid embryos have essentially no reproductive competence (98% lethality rate; 86% miscarriage rate). As a result, there is enough evidence to show that de-selecting uniform aneuploid embryos for transfer based on PGT-A is highly effective. However, not all aneuploidies have the same impact on reproductive potential and gestational outcomes and, as a result, should not be treated equally. In fact, in addition to uniform meiotic aneuploidies, most PGT-A platforms can theoretically reveal mitotic aneuploidies. Mitotic aneuploidies are caused by erroneous chromosomal segregation during post-fertilization mitotic embryonic cell divisions. Mitotic aneuploidies, in contrast to uniform meiotic aneuploidies, are found in a mosaic condition during PGT-A. Mosaicism is defined as the presence of two or more cell lines with different chromosomal content within the same organism. Mosaicism in PGT is commonly designated by the presence of an intermediate chromosome copy number value (e.g., 1.2 or 2.6) for one chromosome or part of it. With the advent of high-resolution methods such as next-generation sequencing, mosaicism has risen to become the third most common cause of "aneuploidies" in PGT-A (up to 20%). Despite this, chromosomal mosaicism in human pregnancies is detected in less than 0.3% of prenatal examinations. Research and clinical studies have shown that mosaicism is very challenging to accurately diagnose from a single TE biopsy and that genetic findings that can be interpreted as mosaicism in PGT-A do not predict reproductive potential. In other words, there is substantial evidence that mosaicism has been largely overestimated in human preimplantation embryos. Furthermore, non-selection studies have revealed that euploid and putative mosaic embryos have similar implantation, miscarriage, and live birth rates. Nonetheless, certain clinics continue to report mosaicism at high rate and to deselect mosaic embryos for in-utero transfer. As a result, the ongoing activities in PGT-A are focused on developing proper outcome measures, explicit frameworks for validating the technology prior to clinical usage, and evidence-based reporting standards.

L 3 Mosaicism in prenatal diagnosis: from NIPT to amniocytes investigation

Malgorzata I. Srebniak
m.srebniak@erasmusmc.nl
Department of Clinical Genetics, Erasmus MC, Rotterdam, The Netherlands

Mosaicism is a common phenomenon in fetal development. The earlier the fetus is investigated the more cases of mosaicism are uncovered. Nowadays the non-invasive prenatal testing (NIPT) allows genotype-first approach, which makes it more difficult to interpret mosaicism. Especially, when mosaicism is encountered in an apparently normal pregnancy. Therefore, this presentation will address current questions on follow up investigations after abnormal NIPT results. Is it possible to offer chorionic villi sampling after an abnormal NIPT result? Is chorionic villi sampling sensitive enough to confirm abnormal NIPT test? A very low mosaicism in in situ amniotic culture used to be interpreted as clinically irrelevant pseudomosaicism and culture artefacts. However, if there is one cell colony found in amniotic fluid showing the same aberrations as found in the non-invasive prenatal test, can you still classify it as pseudomosacism or culture artefact? This presentation will include both fundamental knowledge on mosaicism in prenatal diagnosis and current diagnostic challenges as well.

L4 Hematopoietic Loss of Chromosome Y and Higher Mortality in Men

Lars A. Forsberg
Uppsala Biomedical Centre (BMC), Box 815, SE-751 08 Uppsala, Sweden

Men in the entire world die at younger ages compared with women, on average about 5-6 years. This gender difference is to some degree explained by men's

higher incidence and mortality from several types of common diseases at younger ages compared with women, however, the underlying reason(s) for this male frailty has been elusive. The mosaic loss of chromosome Y (LOY) in blood leukocytes of men help unravelling this long-lasting conundrum in medicine, since men affected with LOY in blood recently have been described to have increased risk for all major causes of human death, including cardiovascular diseases, various forms of cancer and Alzheimer's disease. Using standard technologies such as genotyping arrays, LOY is detectable in more than half of 70+ year old men, and single cell based analyses suggest that this hematological aneuploidy is an inevitable consequence of living, occurring in multiple hematological progenitors of aging men. Moreover, LOY in blood is detectable and associated with disease not only in elderly but also in younger men, and replicated risk factors include increased age, current smoking and genetic background. Hence, as a male specific mutation linked with morbidity and mortality, LOY in blood leukocytes contributes to the reduced lifespan of men, explaining a substantial part of the global sex bias in longevity. However, the full consequences of LOY, its causality and its links with disease etiology in other organs, are topics under current discussion. A series of papers describe that Y loss have various physiological effects on affected immune cells, while also sharing genetic predisposition for disease and genomic instability overall. Intriguingly, recent results from mouse models with LOY show that hematological Y loss causes disease and mortality directly. In addition, it has been described that LOY drives a profibrotic disease mechanism in mice, involving upregulated TGFβ1-signaling by LOY-leukocytes, leading to increased fibrosis in other organs associated with organ failure. In experiments with mice, treatment with TGFβ1-inhibitors reversed the LOY-induced fibrosis and restored organ function. Fibrosis is a process of stiffening and scarring of tissues, in turn associated with exacerbation of various diseases, and is an important component in at least 45% of diseases causing human death. Of note, estimations suggest that as many as 200-400 million young and older men might be affected with LOY globally. Based on observed disease associations and effect sizes, this could translate into 1-4 million male deaths caused by LOY every year. Validating the profibrotic disease mechanism observed in mice, and establishing that LOY drives corresponding processes also in men affected with LOY, could open up for clinical utility. For example, stratification of men based on LOY in blood could enable anti-fibrotic treatments in fields such as cardiology, oncology and geriatrics, using TGFβ1-inhibition in preventive as well as therapeutic purposes.

L 5 Deciphering cancer chromosomal alteration patterns

Sarah McClelland
s.mcclelland@qmul.ac.uk
N. Shaikh[1], A. Mazzagatti[1], M. Guscott[1], L. Tovini[1], S. Johnson[1], B. Bakker[2], D.C.J. Spierings[2], F. Foijer[2], S.E. McClelland*.
[1]Barts Cancer Institute, Queen Mary University of London, EC1M 6BQ, London, UK
[2]European Research Institute for the Biology of Ageing, University of Groningen, University Medical Center Groningen, A. Deusinglaan 1, Groningen 9713 AV, the Netherlands.*

Alterations in chromosomal content and structure are a defining feature of nearly all cancer types. Despite this, very little is known about the mechanisms that cause these aberrations to accumulate, and what their functional significance is to cancer development and therapy resistance. Our lab is interested in defining the mechanisms that cause chromosomal instability in cancer. To do this we take the approach of characterising the acute genomic alterations that occur as a result of specific chromosomal instability mechanisms (for example replication stress, or mitotic dysfunction) using single cell genomics and cell biology. In this way we aim to ultimately track backwards from cancer chromosomal alterations to decipher their origin. In addition, we track the evolution of single cancer cells over time, to determine the rates and types of new genomic alterations that occur at each cell division. Over time we aim to build up a compendium of the mechanisms involved in driving ongoing tumour chromosomal instability, evolution and ultimately therapy resistance in cancer patients.

L 6 Cancer aneuploidy: From evolutionary pressures to cellular vulnerabilities

Uri Ben-David
Ubendavid@tauex.tau.ac.il
Department of Human Molecular Genetics and Biochemistry, Faculty of Medicine, Tel Aviv University

Aneuploidy, an imbalanced number of chromosomes or chromosome arms, is a genetic hallmark of cancer cells, yet aneuploidy remains a biological enigma and a missed opportunity for cancer therapy. My lab combines experimental and computational approaches to dissect the basic biology underlying cancer aneuploidy, to track its origins, and to uncover its cellular consequences. By doing so, we strive to expand our understanding of the genetic basis of cancer, and to make aneuploidy a therapeutic target for cancer treatment.
In this lecture, I will describe two main research areas of my lab:

(1) In the first part, I will discuss how the cellular context affects aneuploidy fitness, and describe

several recent studies in which we assessed the effects of genomic and environmental factors on aneuploidy patterns. These studies provide insights into the selection pressures that shape the evolution of aneuploidy during tumorigenesis, with ramifications for the proper modeling of aneuploidy in cancer research.

(2) In the second part, I will discuss our efforts to identify aneuploidy-induced cellular vulnerabilities in order to selectively kill aneuploid cancer cells. I will focus on a recent large-scale effort to systematically profile and functionally screen isogenic cells with various degrees of aneuploidy, exposing novel therapeutically-relevant cellular vulnerabilities of aneuploid cells.

L7 Optical genome mapping – opportunities for routine cytogenetics and rare disease discoveries

Alexander Hoischen
alexander.hoischen@radboudumc.nl
Departments of Human Genetics and Internal Medicine, Radboud University Medical Center, Nijmegen, The Netherlands

Optical genome mapping (OGM) is an approach to analyze the human genome in particular structural variants (SVs) at a high resolution. OGM offers an opportunity to replace the majority of routine cytogenetic tools, such as karyotyping, FISH and CNV-microarrays for the detection of germline i.e. constitutional and particular somatic i.e. acquired numerical and structural aberrations.
Next to this promise to revolutionize cytogenetics, OGM also harbors potential to identify so far hidden SVs of all kinds in rare disease research. Here I shall present various research examples, including a systematic patient-parent trio study for the systematic assessment of de novo SVs.

L8 Artificial Intelligence in Cytogenetics

Antonio Rausell
Bio-informatique Clinique, Institut Hospitalo Universitaire Imagine, INSERM UMR 1163 Université Paris Cité

Copy number variants (CNVs) are a major cause of rare paediatric diseases. The adoption of whole-genome sequencing as a first-line genetic test has significantly enlarged the load of CNVs identified in single genomes. Together with such increased throughput, clinical interpretation is further challenged by small-size CNVs identified in non-coding genomic regions. In this talk I will present a panel of bioinformatics strategies for the assessment of CNVs in clinical settings recently developed in my laboratory. First, CNVxplorer, a web server suited for the functional interpretation of non-coding CNVs in a clinical diagnostic setting that mines a comprehensive set of phenotypic, genomic, and epigenomic features. Second, CNVscore, a supervised machine learning approach based on tree ensembles and trained on pathogenic and non-pathogenic CNVs from reference databases. Unlike previous approaches, CNVscore couples pathogenicity estimates with uncertainty scores, making it possible to evaluate the suitability of alternative models for the query CNVs. Finally, I will present recent developments on Federated learning (FL) settings that enable multiple institutions to collaboratively train machine-learning models without sharing their local datasets. Here we show that the FL strategies to classify pathogenic and benign variants reached competitive or superior performances as compared to the individual data owners or to the centralized-data model counterparts. Considered together, these developments will highlight the potential and current limitations of novel bioinformatics and Artificial Intelligence-based approaches for clinical cytogenetics applications.

L9 DNA Methylation Episignatures Associated with Large Structural Copy Number Variants: Clinical Implications

Bekim Sadikovic
Verspeeten Clinical Genome Centre; London Health Sciences Centre, London, ON Canada

DNA methylation episignatures are sensitive and specific biomarkers derived from genome-wide DNA methylation profiles in the peripheral blood of patients with a widening range of rare disorders. By utilizing DNA methylation microarrays in combination with EpiSign algorithms, it is possible to simultaneously perform diagnostic screening for over one hundred now-recognized episignature disorders, as well as conduct secondary testing and clinically interpret ambiguous genetic findings in patients with rare disorders. Episignatures are being mapped in a growing number of cytogenetic microdeletion and microduplication syndromes, often providing further insights into the molecular pathophysiology of these disorders. Copy number variation (CNV) microarray analysis is a globally adapted and standardized Tier-I diagnostic assay for screening patients with suspected rare diseases. The integration of CNV and episignature disorder screenings on a single platform would enhance the diagnostic yield, reduce testing redundancy, provide functional interpretation of exceedingly complex genetic variations, and improve health system and patient outcomes. In this presentation, I demonstrate evidence of DNA methylation episignatures in a wide range of CNV disorders and outline an upcoming international project aimed at validating an integrated CNV and EpiSign clinical testing platform.

L 10 Multi Locus Imprinting Disturbance – causes, impact and counselling dilemmas

Karen Temple
ikt@soton.ac.uk
University of Southampton, UK
University Hospital NHS Trust

Imprinting is an epigenomic process, regulated by germline-derived DNA methylation that results in parent of origin specific gene expression. At imprinted loci, epigenetic signatures are laid down in the two separate parental germlines and the pattern is maintained in the genome of the offspring, despite generalised epigenetic reprogramming post fertilisation. There are a relatively small number of these primary imprinted regions (<35) that are differentially methylated, called Imprinting Control Regions (ICRs). They control expression of between 100 - 200 imprinted genes arranged in clusters around them. Through various mechanisms linked to the ICR, imprinted genes are silenced on either their maternal or paternal allele and thus have mono-allelic expression dependent on parent of origin.
'Imprinting Disorders' arise from dysregulation of imprinted gene expression; they are a group of serious congenital conditions that affect metabolism, growth, development and behaviour, and predispose to cancer, significant short stature, obesity and the adult metabolic syndrome. Most of the well-known imprinting syndromes are linked to specific imprinted loci. Mechanisms including copy number variation, uniparental disomy, gene mutations and epimutations result in the syndromes:

- Prader Willi syndrome (PWS), Angelman syndrome (AS) - chromosome 15q11-13
- Beckwith Wiedemann syndrome (BWS), Silver Russell syndrome (SRS) - chromosome 11p15 (also chromosome 7 for SRS)
- Temple syndrome (TS), Kagami Ogata syndrome (KOS) – chromosome 14q32
- Transient neonatal diabetes mellitus (TNDM1) – chromosome 6q24
- Pseudohypoparathyroidism type 1b (PHP1b), Mulchani-Bhoj-Conlin syndrome (MCBS) – chromosome 20q11-13

Multi Locus Imprinting disturbance (MLID) is an epigenetic phenomenon where epimutations occur at several ICRs at the same time, but no agreed definition is yet established. It was first described over 15 years ago in patients with well-recognised imprinting disorders who had additional medical issues or who had more extensive testing. There are few cohorts reported of patients with MLID and no consistency as to which ICRs should be regularly tested for. It remains uncertain whether MLID is a prognostic indicator of a more complex clinical outcome. An increasing breadth of clinical presentations, epigenetic alterations, and genetic trans-acting causes make MLID a complex challenge for clinicians and scientists aiming to diagnose and manage patients.
This talk reflects on what is currently understood about MLID, its causes, medical impact, counselling dilemmas and current testing.

L11 Oligo-based technologies for visualizing nucleic acids

Brian J. Beliveau
beliveau@uw.edu
Department of Genome Sciences, University of Washington, Seattle, USA

Approaches such as genomics, transcriptomics, and proteomics can provide rich information about the presence and abundance of biomolecules in large populations of cells and more recently even in single cells. However, both the ensemble and single-cell versions of these techniques require the dissociation of complex structures like tissues during their experimental workflows, resulting in a loss of spatial information. Multiplexed imaging approaches capable of visualizing multiple DNA, RNA, or protein species in the same sample can provide a valuable complementary approach to the "-omics" methods, particularly in the context of tissues. We have introduced SABER—Signal Amplification by Exchange Reaction. SABER enables the multiplexed amplification of DNA and RNA fluorescent in situ hybridization (FISH) and immunofluorescence signals in fixed cells and tissues, allowing spatial patterns of gene and protein expression and chromosome organization to be mapped in their native contexts. We have also introduced "PaintSHOP", an interactive web-based resource that facilitates the design and ordered of oligonucleotide-based FISH probe sets. Together, these tools provide researchers with an accessible and modular framework for executing spatial imaging experiments.

L12 Complex genomic rearrangements: an underestimated cause of rare diseases

Anna Lindstrand
Anna.lindstrand@ki.se
1. Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden
2. Department of Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden

Complex genomic rearrangements (CGRs) have been known contributors to disease but are often missed during routine genetic screening testing. Identifying CGRs requires 1) detection of copy number variants concurrently with inversions, 2) phasing multiple breakpoint junctions in cis, and 3) detecting and resolving structural variants within repeats. The limitations in detection and resolution of current genetic diagnostic methods may lead to missing or

misinterpreting causal variants, ultimately resulting in suboptimal clinical management.
We demonstrate how combining cytogenetics and new sequencing methodologies can be successfully applied to study the genomic architecture of CGRs. We employ a variety of short- and long-read sequencing technologies to resolve the rearrangement structure and precisely map the breakpoints at the nucleotide level. These results will provide insights to individuals interested in investigating CGRs, accessing their clinical relevance, as well as their impact(s) in rare genetic diseases.

L 13 Distal Germ-Line Deletions in Mosaic With Copy-Neutral Loss of Heterozygosity: Something to Be Considered in Genetic Counselling

Orsetta Zuffardi

The presence of multiple cell lines within the same individual is now considered the rule both in persons suffering from genetic diseases but also in healthy people where the frequency of the mosaic condition was widely demonstrated to increase with aging. Mosaic cell lines may differ for single nucleotide variants and/or structural variants such as copy number gains or losses and copy neutral loss of heterozygosity (CN-LOH).
for years, diseases associated with mosaic conditions were considered de novo and not heritable events. Exemplary is the Proteus syndrome associated with mosaicism for a specific somatic activating mutation in the AKT1 gene. Moreover, healthy people in which the cell line with the disease-variant is present also or only in the gametes have a reproductive risk linked to the percentage of gametes with the disease-variant. In the last ten years a new condition is emerging characterized by unbalanced rearrangements, mainly distal deletions present in healthy or almost healthy persons, in mosaic condition for revertant cell lines in which the imbalance is eliminated thanks to somatic recombination. The new mosaic cell lines are characterized by CN-LoH and segmental uniparental disomy (seg-UPD). The imbalance can be inherited to affected siblings and undergo other revertant events thus creating new mosaic CN-LoH cell lines. In these cases, phenotype-genotype relationship is not predictable a priori, likely depending on how early the revertant lines are formed in embryogenesis and on the possible presence of homozygosity for gene variants within the seg-UPD region. Although the probability of reversion of the original imbalance appears to depend on imbalances of specific genes and is therefore not equal to all genomic variants, it should be taken into account during genetic counseling.

L14 Factors Influencing the clinical expressivity of X-linked inheritance

Brunella Franco
Telethon Institute of Genetics and Medicine (TIGEM); School for Advanced Studies, Genomics and Experimental Medicine Program; Department of Translational Medicine, Federico II University of Naples, Italy

The X-chromosome is peculiar in many ways. In mammals female individuals inactivate one of the two X chromosomes at the early blastocyst stage to achieve gene dosage compensation between sexes. This process, named X chromosome inactivation (XCI) or lyonization, usually occurs randomly. However, in few instances, non-random XCI may take place thus modulating the phenotype observed in female patients carrying mutations in X-linked genes or X-chromosome structural abnormalities. Moreover, it has been estimated that ~25-30% of X-chromosome genes escape XCI. Recent data made available through omics approaches also revealed a high degree of heterogeneity in the levels of XCI among individuals and among different tissues of the same individual.
Several aspects related to dosage compensation contribute to explain the influences of XCI on the phenotypic variability observed in female patients. An overview of the related available data will be presented and clinical examples such as X-linked dominant male-lethal disorders will be used to discuss this intriguing topic.

L15 Structural Variants in Clinical Practice Using Genome Sequencing

Nicolas Chatron
nicolas.chatron@chu-lyon.fr
Genetics Department, Lyon University Hospital, Lyon, FRANCE
Institut Neuromyogène, CNRS UMR 5310 - INSERM U1217, Université de Lyon, Université Claude Bernard Lyon 1, Lyon, FRANCE

The continuous reduction of costs over the last 15 years has permitted a rapid adoption of genomic testing for rare diseases. Exome or genome sequencing are now part of the diagnostic odyssey of rare diseases with a progressive shift towards a first-tier test. The Plan France Médecine Génomique 2025, national genomics initiative, presented in 2016, has now implemented two ISO-accredited clinical labs. Over the first three years, more than 5000 rare disease probands received a result of genome analysis. In this presentation, I will discuss the different barriers that were crossed around structural variant analysis in one of the two labs. First, method validation was performed for Copy Number Variants aiming for non-inferiority towards routine testing (array-CGH or MLPA depending on clinical indication). Then, visualization tools were necessary so that lab geneticists

could judge variant calling accuracy and consider cytogenetic mechanisms. Third, the bioinformatics and interpretation teams progressively adopted Filtering and interpretation rules. Finally, dedicated training sessions and cooperations between cyto-geneticists and molecular geneticists had to be implemented so that chromosomal variants could be properly detected, interpreted and reported. Balanced structural variant analysis was implemented in a second time following the same steps. The overall diagnostic yield is concordant with international publications, 10% of positive cases being structural variants (1% balanced). Final discussion of the presentation will focus on the impact of genome sequencing on cytogenetics diagnostics labs, possible optimizations and future opportunities including alternative sequencing approaches.

L16 Constitutional Chromoanagenesis: From Diagnosis to Genetic Counselling

Caroline Schluth-Bolard
caroline.schluth-bolard@chru-strasbourg.fr
Genetics Diagnosis Laboratory, Institute of Medical Genetics of Alsace, Strasbourg University Hospital, INSERM UMRS_1112, Faculty of Medicine of Strasbourg, Strasbourg France

Chromoanagenesis refers to highly complex chromo-somal rearrangements that were uncovered about ten years ago through the emergence of genome sequen-cing technologies applied to structural variants de-tection. These rearrangements are the result of a single catastrophic event leading to chromosome pulveri-zation and reconstruction in an extremely remodeled derivative.
However, this unifying term hides a more diverse reality in terms of mechanisms, genomic structures or phenotypic consequences.
Chromoanagenesis encompasses chromothripsis, chromoanasynthesis and chromoplexy that each corresponds to a specific mechanism with different genomic consequences. In constitutional diagnosis, chromoanagenesis are identified most of time in patients with abnormal phenotype (intellectual disabi-lity, congenital anomalies) but they are also detected in healthy individuals, mainly after family study. The highly complex rearrangements are usually detected using high-throughput whole-genome approaches such as short-read whole genome sequencing or chromosomal micro-array. However, their complete characterization may often require complementary techniques including Fluorescent In Situ Hybridiza-tion (FISH), long-read sequencing or optical genome mapping. Indeed, these rearrangements may concern one or a few chromosomes, involve small regions or entire chromosomal arms, they may be balanced or unbalanced and may appear on karyotype, often as a more simple rearrangement, or be cryptic. Phenotypic consequences are as diverse as their structure and may be the consequence of genomic imbalances, gene disruption and /or position effect.
Little is known about the meiotic behavior of these highly remodeled derivatives. Such a high complexity is expected to decrease fertility, particularly during male meiosis. Nevertheless, chromoanagenesis trans-missions from parents (mothers and fathers) to the offspring were reported in few cases, either in stable or unbalanced manner, involving chromosome misseggregation or meiotic recombination.
We will illustrate through different examples the chal-lenge of genetic counselling in front of these highly complex and heterogeneous kind of chromosomal rearrangements. We will also discuss the questions raised by these rearrangements regarding chromosome pairing and recombination during meiosis.

L17 Comparative Genomics and Tools for Studying Chromosome Evolution in the Musaceae

Gaëtan Droc [1,2], Guillaume Martin[1,2], Marilyne Summo[1,2], Eloi Durant[3], Alberto Cenci[3], Xue-Jun Ge[4], Pat Heslop Harrison[5], Trude Schwarzacher[5], Nabila Yahiaoui[1,2], Angélique D'Hont[1,2], Mathieu Rouard[3]
[1] CIRAD, UMR AGAP Institut, F-34398 Mont-pellier, France
[2] UMR AGAP Institut, Univ Montpellier, CIRAD, INRAE, Institut Agro, F-34398 Montpellier, France
[3] Bioversity International, Parc Scientifique Agropolis II, 34397 Montpellier, France
[4] Key Laboratory of Plant Resources Conservation and Sustainable Utilization, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510520, China
[5] Department of Genetics and Genome Biology, University of Leicester, Leicester LE1 7RH, UK

Thanks to the advancements in Next-Generation Se-quencing (NGS) technology and the continuous progress made in bioinformatics tools that effectively utilize these technologies, we now could delve deeper in the details of details of how chromosomes have evolved over time. This presentation will focus on tools implemented in the Banana Genome Hub, a crop community portal which facilitates access and study of published genomic information in the Musaceae. The Musaceae family, a member of the order Zingiberales sister of Poales and Palms, encompasses Musa, Ensete, and Musella genera. Within the Musa genus, a classification into two distinct sections exists, Musa and Callimusa. In the Callimusa section, the predomi-nant basic chromosome number is x = 10; however, certain species like M. beccarii display a reduced count of x = 9, which is shared with its sister genera Ensete and Musella. Conversely, Musa exhibits a consistent basic chromosome number of x = 11, but their chromosomes have been shaped by complex processes of inter and intraspecific hybridizations as well as reciprocal translocations. The Banana Genome Hub incorporates provide access to graphical inter-

faces for comparative analysis, such as a synteny viewer for comparing genome structures across chromosome-scale assemblies. Additionally, new interfaces were implemented to explore genome ancestry mosaics in cultivated bananas, translocations and pangenomes, contributing thus to the study of chromosome evolution.

L18 Coleopteran Satellite Profiles: Chromosomal and Sequence Organization

Brankica Mravinac
brankica.mravinac@irb.hr
Ruđer Bošković Institute, Division of Molecular Biology, HR-10000 Zagreb, Croatia

Satellite DNAs (satDNAs) are highly repetitive DNA sequences whose tandemly arranged repeat units form very long arrays primarily in the non-coding regions of eukaryotic chromosomes. They have traditionally been considered dominant components of heterochromatin blocks, but with the advent of whole-genome sequencing and genome projects, the locations of satDNAs throughout the genome, including euchromatic regions, have been increasingly discovered. Nevertheless, the study of these sequences has always been challenging. Their repetitive organization has severely hindered or even prevented the correct assembly of satDNA arrays, resulting in their omission or drastic underestimation in genome assemblies. Even with ultra-long read sequencing paving the way for gapless genomes, satDNAs still remain the genome bastions that resist accurate assembly. Coleopteran insects of the genus Tribolium are important stored product pests, but they are also an excellent system for studying satDNAs. A quarter century ago, it was discovered that Tribolium species are dominated by one or two species-specific satDNAs that make up 20-40% of their genomes. In this talk, the new findings on the Tribolium satDNA profiles will be presented. Three decades after the first satDNAs were described in these species, long-read sequencing reveals that over a hundred additional satDNAs are hidden in their genomes. By combining in silico and in situ approaches, we decipher their sequence and chromosomal organization, detecting intense rearrangement dynamics even in heterochromatin regions. By comparing the satellitomes, the satDNA collections, from closely and distantly related Tribolium species, we disclose species-specific satDNAs as well as those conserved at the genus level. In this sense, sibling species that can still hybridize but produce sterile F1 hybrids are particularly informative. Through a comparative analysis of satDNAs located in the (peri)centromeric regions of the siblings, we attempt to get close to answering the question of whether satellites may play a role in postzygotic reproductive isolation. The new findings also challenge the earlier assumption that the most abundant satDNAs are key components of functional centromeres. Although we cannot yet answer all of these questions with certainty, by studying Tribolium satellite profiles we are learning a great deal about the genomes of the important coleopteran pests, but also about the chromosome biology of non-model organisms.

L19 Epigenetic regulation of genome architecture in development and cell differentiation

Giacomo Cavalli[1,2]
[1] Institute of Human Genetics, CNRS, 34396 Montpellier Cedex 5, France
[2] University of Montpellier, Montpellier, France

The eukaryotic genome folds in 3D in a hierarchy of structures, including nucleosomes, chromatin fibers, loops, chromatin nanodomains, topologically associating domains (TADs), compartments and chromosome territories that are highly organized in order to allow for stable memory as well as for regulatory plasticity, depending on intrinsic cues, such as chromatin association of Polycomb proteins, CTCF and cohesion, and on environmental cues. We showed that TADs and chromatin loops can assist gene regulation, both in *Drosophila* and in mouse cells. However, the physical nature of compartments, TADs and loops remain elusive and single-cell studies are critically required to understand it. We characterized chromatin folding in single cells using super-resolution microscopy, revealing structural features inaccessible to cell-population analysis. TADs range from condensed and globular objects to stretched conformations. Favored interactions within TADs are regulated by cohesin and CTCF through distinct mechanisms. Furthermore, super-resolution imaging revealed that TADs are subdivided into discrete nanodomains.
We also analyzed chromatin loops. Ultra-high resolution analysis of genome architecture by microC shows that a multitude of specific chromatin contacts are formed, of either architectural, activating or repressing function. We studied functionally loops that depend on factors that regulate the expression of a large number of genes, dubbed as Polycomb group proteins. Originally, they were shown to silence gene expression and we found that they induce the formation of chromatin loops. The disruption of one of these loops reduces silencing of a target genes, suggesting that loops may play instructive roles in gene regulation. Surprisingly, Polycomb components are also involved in chromatin loops linked with transcriptional activation. Furthermore, their chromatin organization ability can induce stable epigenetic memory that can be inherited through cell division and across organismal generations. Our progress in these fields will be discussed.

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L20 Transcription shapes spatial organization of eukaryotic genes

Irina Solovei
Biocenter, Ludwig Maximilians University Munich, Planegg-Martinsried, Germany

Despite the well-established role of nuclear organization in the regulation of gene expression, little is known about the reverse: how transcription shapes the spatial organization of the genome. In particular, given the limited resolution of light microscopy, small size of genes and chromatin folding in the nucleus, the structure and spatial arrangement of a single transcribed gene is poorly understood.

We made use of several long highly expressed mammalian genes and demonstrated that they form Transcription Loops with polymerases moving along the loops and carrying nascent RNAs that undergo co-transcriptional splicing. Transcription loops dynamically modify their harboring loci, separate their flanks and extend into the nuclear interior. Both experimental evidence and polymer modeling suggest that transcription loops formed by highly expressed genes acquire an intrinsic stiffness caused by the dense decoration of gene bodies with multiple polymerases carrying voluminous nascent RNPs.

Our finding rules out a popular hypothesis about eukaryotic transcription occurring in so-called Transcription Factories presumably formed by immobilized polymerases, to which transcriptionally active genes are pooled and reeled through extruding nascent and mRNAs in a limited spot. Although we demonstrated the transcription loop formation for long highly expressed genes, we propose that it is the universal principle of eukaryotic gene expression, which has not been appreciated before due to small size or low expression of studied genes and limited microscopy resolution.

L21 Adding a chromosome perspective to plant genomics: making sense of retained retroviruses, moving retrotransposons and expanding satellite DNAs

Tony Heitkam
Technische Universität Dresden, Fakultät Biologie, Dresden

In the past, plant cytogenetics has profited immensely from the identification of repetitive DNAs. Due to their abundance in the genome, repetitive DNAs are excellent cytogenetic probes, with the potential to uniquely label certain chromosomes and chromosomal regions. But in the face of advancing genomics, does this technique have a time stamp? Using examples from the lab's current research into plant genome evolution and plant breeding, this talk aims to address the two questions:

(1) What are use cases, in which straight-forward *in situ* hybridizations of repetitive DNA probes can provide most insights? – I argue that repeat-based cytogenetics is especially valuable to understand the chromosomal basis of emerging crops and their wild relatives.

(2) How can cytogenetics strengthen any genomic endeavor? – Here, I argue that the applications are plentiful: (i) Understanding the polyploid heritage of a crop, (ii) gaining a deep view into heterochromatic, underrepresented genomic regions, and (iii) assessing chromosomal and genomic stability are just some of the new applications that are powered by genomics.

Taken together, I use examples from our recent research in plant genomics – spanning satellite DNAs, retrotransposons and viral relics – to illustrate how genomics and cytogenetics can benefit from each other.

L22 The new ISO 15189 standard for medical laboratories

Folker Spitzenberger
University of Applied Sciences Lübeck, Mönkhofer Weg 239, 23562 Lübeck
Fraunhofer Research Institution for Individualized and Cell-Based Medical Engineering - IMTE, Mönkhofer Weg 239a, 23562 Lübeck
folker.spitzenberger@th-luebeck.de
folker.spitzenberger@imte.fraunhofer.de

The standard ISO 15189 is internationally regarded as the gold standard for quality and competence in medical laboratories and is the basis for the accreditation of these laboratories. The recent revision from 2022 has been met with controversy: on the one hand, an adjustment to the state of the art was necessary, on the other hand, there are many new aspects that can be challenging to implement. The lecture examines the content and implementation concepts for this standard.

ISO 15189:2022 is a work result of the ISO Technical Committee 212 "Clinical laboratory testing and in vitro diagnostic test systems" and was developed within more than four years by over 50 international experts under Canadian leadership as Revision No. 4 of ISO 15189.

The focus of the structural and content-related amendment of ISO 15189 is the adaptation to the current version of the ISO/IEC 17025 standard, which represents a so-called normative reference for ISO 15189. Elements of ISO 9001:2015 also form the basis for adapting the requirements for the documentation of the management system and for the opportunity and risk management of medical laboratories. However,

due to the special requirements in the sensitive area of medical laboratory diagnostics, risk management goes far beyond the generic requirements of ISO 9001 and ISO/IEC 17025.
New requirements also arise regarding the impartiality and confidentiality of medical laboratories.
Within the framework of medical-scientific competence, extended requirements for the implementation of internal quality control of examination procedures and for the selection, implementation and evaluation of EQA programs form a further focus of the standard. With regard to information management in medical laboratories, aspects of cybersecurity and risk management in emergency situations are brought to the fore.
The standard basically extends its scope to the POCT area, which means that the previous POCT standard ISO 22870 will lose its validity after the end of the three-year transition period of ISO 15189:2022 (December 5, 2025).

L23 HGVS nomenclature: recommendations to describe variants in DNA, RNA and protein sequences

Johan T. den Dunnen
representing the HGVS Variant Nomenclature Committee (HVNC), a HUGO committee

To allow accurate sharing of information of variants in the genome, we need a standard which is used world-wide. THE standard developed for the description of variants detected using microscopic techniques has been developed by the Standing Committee on Human Cytogenomic Nomenclature (the ISCN standard). THE standard developed for the description of variants detected at the nucleotide level has been developed by the Human Genome Variants Society (the HGVS nomenclature standard).
The HGVS nomenclature standard originates from the activities of a group of scientist interested in the collection of disease-causing variant, the HUGO Mutation Database Initiative (HUGO-MDI). Nowadays the recommendations are under the auspices of the Human Genome Organisation (HUGO). A committee with expert representatives elected from diverse groups of users, the HGVS Variant Nomenclature Committee (HVNC), collects all requests for modifications/additions of the standard, decides whether changes are required and if so prepares a suggestion for every body to give comment s(the Community Consultation step, open for 2 months). All comments will be collected, discussed in the HVNC and a decision made. All details regarding HGVS nomenclature are available through the nomenclature website: varnomen.HGVS.org (*current version 20.05*). Where necessary the pages will be modified to correct errors, to clarify issues (e.g. by adding examples) or updated when new recommendations have been approved.
The ISCN and HVNC work closely together, trying to harmonize recommendations from both committees and prevent undesired differences. However, as always, history plays a role and not all recommendations are identical. In my presentation, after introducing the basics of HGVS nomenclature, I will focus on the differences between the ISCN and HGVS. When describing variants, the rule of thumb to remember is that when microscopic data are available you use the ISCN standard, when you have sequence data you use the HGVS standard.

L24 The test performance of the non-invasive prenatal test in vanishing twin pregnancies and multiple gestations

Robert-Jan H. Galjaard, van Eekhout J.C.A., Bax C.J., Bekker M.N., Dutch NIPT consortium

Objective: To evaluate the test performance of the non-invasive prenatal test (NIPT) in multiple gestations and in vanishing twin pregnancies.
Methods: This study is part of the TRIDENT-2 study where NIPT is offered as a first-tier test to women with a multiple gestation or vanishing twin between July 1, 2020, and April 1, 2023. Data for this abstract was collected until January 1, 2023. NIPT was confirmed by follow-up prenatal invasive testing or postnatal genetic testing. Chorionicity and amnionicity were determined by first-trimester ultrasound.
Results: Until January 1, 2023, NIPT was performed in 3755 pregnant women with a multiple gestation and in 598 vanishing twin pregnancies. Among 2687 dichorionic-diamniotic twin gestations, the NIPT tested positive for trisomy 21, 18 and 13 in 0.78% (n=21), 0% (n=0), and in 0.11% (n=3) respectively. for trisomy 21 the sensitivity was 100% (95% CI 83-100%), the specificity was 100% (95%CI 99.9-100%), and the positive predictive value (PPV) 100% (95% CI 83-100%). None of the trisomy 13 cases could be confirmed. In the 994 monochorionic twin gestations, NIPT tested positive for trisomy 21 in three cases, which were all confirmed. Among 598 vanishing twin pregnancies the NIPT tested positive for trisomy 21, 18 and 13 in 2.7% (n=16), 0.67% (n=4) and 1.2% (n=7) respectively. Six trisomy 21 cases could be confirmed in the developing fetus: sensitivity 100% (95%CI 54-100%), specificity 98% (95%CI 97.3-99.4%), PPV 43% (95%CI 17-71%). No discordant negative results were reported.
An additional finding was detected in 0.43% (n=17) of the multiple gestations and in 4.35% (n=26) of the vanishing twin pregnancies.
Conclusion: Currently available data show that NIPT is able to accurately predict trisomy 21 is multiple gestations and vanishing twin pregnancies. For trisomies 18 and 13 this might be less accurate.

L25 Liquid biopsy in reproductive medicine

Joris Vermeesch
joris.vermeesch@uzleuven.be
Laboratory of cytogenetics and genome research, Department of Human Genetics, Leuven
Non-invasive sampling of an individual's body fluids is an easy means to capture circulating cell-free DNA (cfDNA). These small fragments of DNA carry information on the contributing cell's genome, epigenome, and nuclease content. Analysis of cfDNA for the assessment of genetic risk has already revolutionized clinical practice, and a compendium of increasingly higher-resolution approaches based on epigenetic and fragmentomic cfDNA signatures continues to expand. Profiling cfDNA has unlocked a wealth of molecular information that can be used for prenatal diagnosis. I will present how we use cfDNA analyses for the diagnosis of the fetus, the pregnant mother and explore new avenues for liquid biopsy.

L26 Delivering a national fetal exome sequencing service: Diagnostic yield, challenges and pitfalls.

Lyn Chitty.
l.chitty@ucl.ac.uk
North Thames Genomic Laboratory Hub, Great Ormond Street NHS Foundation Trust and Genetics and Genomics, UCL Great Ormond Street Institute of Child Health.

In 2020 NHS England established a rapid fetal exome sequencing service to improve the identification of monogenic conditions in fetuses with abnormalities suggestive of a monogenic aetiology. Referral is through clinical genetics after a multidisciplinary discussion with fetal medicine specialists and clinical scientists. This is a national service that to date has sequenced more than 700 cases with a diagnostic yield of around 30% with a turnaround time of less than two weeks. In this presentation some of the more challenging cases will be discussed to illustrate the issues we have faced, including poor phenotyping, identification of new variants, incidental findings with strategies for how we have managed these.

L27 The Landscape of Structural Variation Across Diverse Global Populations and Developmental Disorders

Michael E.Talkowski

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Abstracts - Oral Presentations

O1 Normal array CGH results in a patient with short stature and global developmental delay carrying a de novo ring chromosome 2p and a chromosome 2q derivative with a neocentromere

Cornelia Daumer-Haas, Daniela Koehler, Eva Kahles, Niklas Hirschberger, Sabine Minderer, Karl-Philipp Gloning
Eurofins, Humangenetik und Praenatal-Medizin MVZ Gmbh, Munich-Germany

A 3-year-old patient presented with microcephaly, short stature (p3) and developmental delay especially regarding speech. There were no dysmorphic abnormalities. Because of his aberrant behavior autism spectrum disorder was suspected.

Fra(X) analysis, array-CGH and whole exome sequencing showed normal results. Surprisingly the diagnosis was made by conventional karyotype analysis revealing a complex karyotype with 47 chromosomes in most of the cells: A ring chromosome 2p and a derivative chromosome 2q with a neocentromere formation. FISH analysis showed the presence of subtelomere 2p on the 2q derivative, which had a normal subtelomere 2q and no signal with the 2 centromeric probe which was present in the ring chromosome. Karyotype (ISCN 2020):

mos 47,XY,-2,+r(2p),+neo(2)[26]/46,XY,-2,+neo(2)[4]/47,XY,-2,+?dic r(2p),+neo(2)[1].ish r(D2Z1+), neo(2)(VIJyRM2052+,D2S447+) de novo.

We could identify cells without a ring and a cell with a large, likely dicentric ring as described in other ring chromosome cases. The abnormal cell lines pointing to mitotic instability are probablyresponsible for the phenotype of our patient ("Ring syndrome").

The normal array-CGH and whole exome analysis results indicate no deletion/duplication in the complex karyotye and no monogenetic cause of the phenotype. Severe growth deficiency, developmental delay and aberrant behaviour without major malformations is a common finding in a ring chromosome carrier. Precise genotype-phenotype correlations for ring chromosomes may not be possible, since influencing factors vary depending on ring instability and the level of mosaicism. (Moh-Ying et al. Review Translational Pediatrics 2015).

No comparable case with a de novo event leading to a ring chromosome of the short arm of chromosome 2 and a derivative 2q with a neocentromere could be found in the literature.

With array-CGH and whole exome analysis alone, the diagnosis would have been missed.

O2 Multicentric longitudinal performance monitoring of different non invasive prenatal screening technologies used in Belgium

Armelle Duquenne[1], Axel Marichal[2], Machteld Baetens[3], Bettina Blaumeiser [4], Nathalie Brison [5], Marie Bruneau [6], Sakia Bulk [7], Bert Callewaert [3], Sandra Coppens [6], Michelle Deberg [7], Anne De Leener [1], Marjan Derademaeker [4], Julie Desir [8], Koenraad Devriendt [5], Annelies Fieuw [9], Jean Stephane Gatot [7], Mauricette Jamar [7], Katrien Janssens [4], Sandra Janssens [3], Colombine Meinier [8], Ilse Parijs [5], Bruno Pichon [6], Sonia Rombout [8], Ileen Slegers [9], Yves Sznajer [1], Olivier Vanakker [10], Kim Van Berkel [9], Leen Van Coillie[5], Ann Van Den Bogaert[9], Isabelle Vandernoot[11], Kris Van Den Bogaert [5], Joris Vermeesch [5]
University Hospital Louvain - Uclouvain, Center for Human Genetics, Brussels-Belgium [1] Nstitut De Pathologie Et De Génétique - Ipg, Center for Medical Genetics, Gosselies-Belgium [2] University Hospital Ghent, Center for Medical Genetics, Ghent-Belgium [3] University Hospital Antwerp, Center for Medical Genetics, Antwerp-Belgium [4] University Hospitals Leuven–ku Leuven, Center for Human Genetics, Leuven-Belgium [5] University Hospitals Erasme - Ulb, Center for Human Genetics, Brussels-Belgium [6] University Hospitals Liège, Center for Medical Genetics, Liège-Belgium [7] Institut De Pathologie Et De Génétique- Ipg, Center for Medical Genetics, Gosselies-Belgium [8] University Hospital Brussels - Vub, Center for Medical Genetics, Brussels-Belgium[9] University Hospital Ghent, Center for Human Genetics, Ghent-Belgium [10] University Hospitals Erasme - Ulb, Center for Medical Genetics, Brussels-Belgium[11]

Objectives

Belgium was the first country to fully reimburse noninvasive prenatal screening (NIPS) as a nationwide first-tier screening test for all pregnant women. Different commercial and in-house developed NIPS technologies are being used. Although the accuracies (sensitivity, specificity, positive predictive value, negative predictive value) of those tests are published and provided on the websites of the different providers, longitudinal studies to monitor and compare the performance of those methods are lacking. Since all invasive prenatal genetic testing following positive NIPS are analyzed at one of eight Belgian genetic centers, we are uniquely positioned to determine the performance of different NIPS technologies.

Method

All invasive tests performed following a positive NIPS between 01/07/2019 and 30/06/2022 were registered. Both the NIPS technology used and the results of the diagnostic tests were aggregated. We provide the

distribution and the positive predictive values of the different trisomies per technology.
Results
1128 diagnostic assays were performed from 01/07/ 2019 to 30/06/2022 following a positive NIPS for the common trisomies 13, 18 and 21. of these NIPS, 59% (#680), 15% (#156), 7% (#75) and 5% (#58) were respectively done by a LDT method, VeriSeq® (Illumina), Harmony®(Roche) and Vanadis®(Perkin Elmer). for 14%, we could not determine the NIPS technology. for T21, the actual PPVs for LDT, VeriSeq®, Harmony® and Vanadis® are respectively 92%, 75%, 92% and 69%.
Conclusions
This longitudinal study more-or-less confirms the reported accuracies. The LDT method outperforms most commercial offerings. Although small false positive rates for all, the small differences in performance significantly impact pregnant women and the health care system. With 120000 pregnancies/year in Belgium and an incidence of 0.3% for T21, a PPV of 69% versus 92% corresponds to a yearly increase of unnecessary invasive tests from 28 to 112.

O3 Optical Genome Mapping for multiple myeloma evaluation of the technology in a clinical laboratory.

Christina Srouji[1], Nivin Moustafa-Hawash[1], Noa Lavi[2], Karin Weiss[1], Tamar Paperna[1]
Rambam Health Care Campus, Genetic Institute, Haifa-Israel[1] Rambam Health Care Campus, 2department of Hematology and Bone Marrow Transplantation, Haifa-Israel[2]

Background:
Cytogenetic tests for multiple myeloma (MM) consist of a series of FISH analyses on enriched CD138+ cells from bone marrow aspirates (BMA). Optical genome mapping (OGM) is a new genome-wide cytogenetic technology, whose use in MM is limited by the number of cells obtained from magnetic beads remnants in the enriched fractions.
Objective:
To evaluate OGM as an alternative for FISH tests in MM and compare outcomes of CD138+ fraction versus direct BMA analysis in samples with >25% MM cells by immunophenotyping.
Methods:
BMA was obtained from MM patients at diagnosis. CD138+ cell fractions were enriched using MACS-prep MM CD138+ Microbeads and processed with routine FISH analyses. OGM analyses were done on DNA extracted from CD138+ fractions, and for some samples, when the MM fraction was >25% by immunophenotyping, also directly from BMA. Rare variant analysis was employed for all samples. OGM results were compared with FISH results.
Results:
MM samples required modification of the DNA extraction protocol to ensure the removal of remnant magnetic beads. OGM analyses detected all chromosomal aberrations previously reported by FISH tests. In most cases, OGM identified additional structural variations, including gains, losses, and translocations. When immunophenotyping indicated >25% MM in BMA, we observed that all the structural aberrations detected in enriched CD138+ analysis were also detected by BMA analysis for the same patient, albeit at lower VAFs
Conclusions:
OGM demonstrated high concordance with routine FISH testing and revealed new aberrations that may be relevant to diagnosis and therapeutics. OGM is an exciting new technology that can potentially replace current cytogenetics tests as a first-tier test for MM and other hematology diagnoses. An expanded evaluation of the direct use of OGM on BMA in cases of high CD138+ fraction is merited.

O4 Dam Assisted Fluorescent tagging of Chromatin Accessibility (DAFCA) for optical genome mapping in nano channel arrays

Gil Nifker [1]
Tel Aviv University, Physical Chemistry, Tel Aviv-Israel[1]

Chromatin accessibility is a central epigenetic regulator of gene expression, and its genomic profile holds essential information on the cell type and state. We utilized the E.coli Dam methyltransferase in combination with a fluorescent cofactor analogue to generate fluorescent tags in accessible DNA regions within the cell nucleus. The accessible portions of the genome are then detected by single-molecule optical genome mapping in nano-channel arrays. This method allowed us to characterize long-range structural variations and their associated chromatin structure. We show the ability to create whole-genome, allele-specific chromatin accessibility maps composed of long DNA molecules extended in silicon nano-channels. Common methods for assessing chromatin accessibility such as ATAC are based on short-read next generation sequencing (NGS),and suffer from the inherent limitations of short-reads. Specifically, short-read sequencing is limited in its capacity to detect genomic structural variations (SVs) and copy number variations (CNVs) such as those in large repetitive elements. Such variations have a direct impact on genetic disorders and cancer, with established structural aberrations associated with specific disease. The need to resolve large scale genetic structure has driven developments in cytogenetic technologies such as karyotype, fluorescence in-situ hybridization (FISH), and arrays for comparative genomic hybridization (CGH. Optical Genome Mapping (OGM) is the most advanced cytogenetic technology, enabling full characterization of SVs and CNVs at high resolution.

O5 Triploid conceptions are predominantly caused by female meiosis II errors and their risk increases with advancing maternal age

Ludovica Picchetta [1], Matteo Figliuzzi [1], Maurizio Poli [1], Yiping Zhan [2], Xin Tao [2], Chaim Jalas [2], Silvia Caroselli[1], Antonio Capalbo[1]
Juno Genetics Italia, Reproductive Genetics, Rome-Italy [1] Juno Genetics Usa, Reproductive Genetics, Basking Ridge, New Jersey-United States[2]

Rationale: Normal fertilization is denoted by the appearance of 2 pronuclei (2PN) 16-18 hours after insemination. Deviation from 2PN is considered evidence of abnormal fertilization and ploidy anomalies. Nonetheless, even 2PN embryos can be diagnosed with ploidy abnormalities during preimplantation genetic testing (PGT).
Study Design: This is a retrospective study including 96,660 trophectoderm biopsies analyzed between 2020 and 2022 via a targeted NGS based PGT platform. Parental and meiotic origins were estimated using two independent approaches: (i) using sex chromosomes CN from 1,063 embryos with altered ploidy status (ii) using genotyping data from 57 trios (embryo+parents) from PGT-M cycles where also genome-wide recombination events were investigated.
Results: The prevalence of ploidy abnormalities in 2PN-derived embryos was 1.1% (n=1,063/96,660), with triploids accounting for 83.0% (n=882/1,063) and haploids for 17.0% (n=181/1,063). Remarkably, the incidence of triploidy was positively correlated with maternal age (OR=1.059 per year; p<0.001). Based on sex chromosomes CN analysis, the extra haploid set of triploid embryos was almost completely of maternal origin (94.6%; 95%CI:93.0-96.1), with male errors accounting for only 5.4% (95%CI:4.0-7.1). Haploid embryos were the result of paternal errors in 97.8% of cases (95%CI:94.4-99.4). In terms of triploidy's meiotic origin, two-thirds of the errors occurred during MII (95%CI:63.4-69.8), while one-third occurred during MI (95%CI:30.2-36.5). Using genotyping data of 57 trios we confirmed the predominance of paternal error in haploidy (n=12/14) and the maternal origin of embryonic triploidies (n=43/43). The extra haploid set resulted from an error during MI in 27.9% (n=12/43) and during MII in 72.1% (n=31/43) of cases. Interestingly, 16.3% of triploids (n=7/43) showed no genome-wide recombination events.
Conclusion: Thanks to the exceedingly high sample size, this is the first study to reveal an increased risk of triploid conception with advancing female age (76% higher at age 40 than at age 30), providing meaningful insights for patients counseling. Importantly, the parental and meiotic origin of ploidy anomalies in embryos were unveiled.

O6 Multiomic profiling unravels disease mechanisms in complex chromosomal rearrangements and marker chromosome carriers

Marlene Ek[1], Kristine Bilgrav Saether[1], Jakob Schuy[1], Claudia M B Carvalho[2], Jesper Eisfeldt[1], Anna Lindstrand[1]
Karolinska Institutet, Department of Molecular Medicine and Surgery, Stockholm-Sweden [1] Pacific Northwest Research Institute, Department of Molecular and Human Genetics, Seattle, Wa-United States [2]

In clinical cytogenetic laboratories, chromosomal rearrangements may warrant further analysis by genomic technologies. Such events can vary in size and include both gross chromosomal events, such as translocations, inversions and supernumerary ring/marker chromosomes, as well as small deletions or duplications. In order to fully understand the complexity of a specific event, infer underlying mechanisms and clinical consequences, breakpoint junctions need to be pinpointed and the derivative chromosome structure resolved.
We have utilized combinations of short-read, linked-read and long-read genome sequencing to characterize DNA samples from over 50 carriers including inversions, translocations, rings and markers as well as complex chromosomal rearrangements. By multiomic analysis of those cases, we have been able to identify disease causing genes and other disease mechanisms such as disturbed long-range interactions. Furthermore, the detailed analysis of breakpoint junctions show that different mutational processes may cause similar rearrangements such as both translocations and inversions being generated from either replicative and non-replicative mechanisms. Finally, we show that chromosomal rearrangements often involve more complexities than the cytogenetic investigations indicate, such as a marker chromosome with material from chromosome 7, X and 5.

O7 Long read whole genome sequencing for the detection of structural and epigenetic variation in developmental disorders

Mathilde Geysens[1], Benjamin Huremajic[1], Julio Finalet[1], Nicolas Dierckxsens [1], Greet Peeters [1], Erika Souche [1], Joris Vermeesch [1], Kris Van Den Bogaert [1]
Kuleuven, Human Genetics, Leuven-Belgium[1]

Technological improvements over the last decades have been instrumental for the identification of numerous chromosomal anomalies and monogenetic diseases, shedding light on the extreme genetic heterogeneity underlying developmental disorders. Despite the associated significant increase in diagnostic yield, about 60% of patients remain without molecular diagnosis. There is growing evidence that cryptic structural variations and methylome anomalies are an important cause of hitherto unsolved cases.

Promising recent reports indicate that this diagnostic gap could be bridged by long-read sequencing platforms. The broad adoption of these methods has long been hindered by their high costs and error rate but should become reality as both, costs and error rates, keep dropping.
Further research is however needed to evaluate the clinical relevance of this new technology and enable its implementation in a diagnostic setting. To address these objectives, we perform trio whole genome nanopore sequencing in 50 patients with intellectual disability and/or multiple congenital anomalies without molecular diagnosis after short read whole exome or genome sequencing. We developed an analytical pipeline to assess coding and non-coding structural variants as well as methylome disturbances. As a proof-of-concept, we first evaluated the detection of structural variants, short tandem repeats, repeat associated methylation disturbances (FMR1, FSHD) as well as differential methylation of disease associated loci (11p15.5, 14q32 and 15q11q13) and episignatures in controls with a known disorder. The assessment of the added value of the technology in 50 unsolved patients as well as the construction of the essential population reference of normal structural variation is ongoing.
We will present our project, the results of the proof-of-concept phase as well as the first results, perspectives and challenges in the unsolved cohort. We envision a significant increase in the number of patients that will receive a molecular diagnosis, improving the understanding of molecular disturbances underlying diseases and patient care.

O8 Educational benefits of analysing highly complex chromosomal rearrangements such as chromoanagenesis by long read approaches

Mathilde Quibeuf [1], Mercati Oriane [1], Kévin Cassinari [1], Anne-marie Guerrot [2], Claude Houdayer [2], Géraldine Joly-Helas [1], Pascal Chambon [1]
Normandie Univ, Unirouen, Inserm U1245, CHU Rouen, Fhu G4 Génomique, Department of Genetics and Reference Center for Developmental Disorders, Rouen, F, Cytogenetics Laboratory, Department of Genetics, Rouen-France [1] Normandie Univ, Unirouen, Inserm U1245, CHU Rouen, FHU G4 Génomique, Department of Genetics and Reference Center for Developmental Disorders, Rouen, F, Department of Genetics, Rouen-France [2]

Introduction. The detection of structural variants by long-read approaches is one of the keys to the management of patients in diagnostic deadlocks. This work will show how the analysis of a highly complex chromosome rearrangement, such as a chromoanagenesis, helps to acquire new benchmarks and new interpretation logics specific to the different types of alterations that are now detectable by these techniques, especially by Optical Genome Mapping (OGM).
Patient and results. Chromoanagenesis was identified in a 3-month-old girl presenting with mild dysmorphic features, minor malformations and a significant motor delay. This diagnosis was based on (i) the results of array-CGH showing a 8,3Mb 6q15q16.3 deletion, associated with seven smaller deletions with poor or no gene content and restricted to a limited number of chromosomes and (ii) the analysis of the karyotype showing an additional complex translocation between chromosomes 5, 6 and 12. Long read analysis was performed by OGM for further characterization of the anomaly. Interestingly, OGM data helped new users to apprehend the different algorithms and tools. New users were guided to retrieve (i) all the CNVs detected by array CGH, (ii). the translocation identified by karyotyping, and also (iii) additional balanced structural variants, such as a paracentric inversion, intrachromosomal insertions and the combination of insertion and translocation. Both the graphical interface and the raw text result files were needed to fully characterize the chromoanagenesis.
Discussion. This work illustrates the importance of analyzing a complex chromosomal anomaly such as chromoanagenesis in order to quickly apprehend the whole spectrum of anomalies identifiable by long read approaches. Sharing such data on complex rearrangements could help the community to improve the understanding and the use of long-read approaches.

O9 Burden of long range position effects in balanced chromosomal rearrangements

Niels Tommerup [1], Chelsea Lowther[2], Mana Mehrjouy[1], Malene B. Rasmussen[3], Iben Bache [4], Mads Bak [4], Ryan L. Collins[2], Harrison Brand[2], James F. Gusella[2], Cynthia C. Morton[5], Michael E. Talkowski[2]
University of Copenhagen, Department of Cellular and Molecular Medicine, Copenhagen-Denmark[1] Massachusetts General Hospital, Center for Genomic Medicine, Boston, Ma-United States[2] Danish National Genome Center, Department of Healthcare, Copenhagen-Denmark[3] Copenhagen University Hospital, Rigshospitalet, Department of Clinical Genetics, Copenhagen-Denmark[4] Brigham and Women's Hospital, Department of Obstetrics and Gynecology, Boston, Ma-United States[5]
Danish Cytogenetic Central Registry Study Group*, International Breakpoint Mapping Consortium (IBMC)*, Developmental Genome Anatomy Project (DGAP)*
*Full author list at
https://www.medrxiv.org/content/10.1101/2022.02.15.22270795v1

The risk of early onset developmental disorders (DD) in carriers of balanced chromosomal rearrangements (BCRs) is estimated to be 26.8% due to direct gene truncation or long range position effects (LRPE) (Halgren 2016). In a systematic study of >700 simple two-way affected and healthy BCR-carriers, 21.3% of the BCRs directly disrupted a known autosomal

dominant DD gene. We identified an enrichment of noncoding BCR breakpoints in specific topological domains (TADs) involving both known (e.g. SOX9, FOXG1) and novel LRPE loci (BCL11A, BCL11B) that accounted for 7.1% of the affected cohort. However, manual curation revealed a further 2% of cases with breakpoints truncating TADs known to be associated with dysregulation of specific DD genes by LRPE. We identified six genomic features enriched in TADs preferentially disrupted by noncoding BCRs in affected cases versus controls and used these features to build a model to predict TADs at risk for LRPEs across the genome. The top predicted TADs harboured evolutionary conserved elements as well as known DD associated genes. LRPE-associated breakpoints are either located 5´ or 3´ to the specific dysregulated gene (breakpoint polarity) which is explained by the local chromation organization. Breakpoints in cases were not enriched on any particular chromosome, except for chromosome 14, where a significant excess was due to breakpoint clustering within five TADs, that include known (FOXG1, BCL11A, BCL11B) and novel (LRFN5 and FLRT2) disease genes and LRPE loci. Intruigingly, we observed a significant excess of cases where both breakpoints truncated a predicted high-risk TAD, suggesting that dual loss/gain of regulatory elements in two high-risk TADs may be a common morbidity mechanism (enhancer swapping/shuffling/ adoption). Conservatively, we estimate that LRPE may be at least as frequent a mechanism underlying early DD in BCRs as direct gene truncation.

O10 3D nuclear architecture distinguishes thyroid cancer histotypes

Aline Rangel-Pozzo [1], Filipe F Dos Santos [2], Tinuccia Dettori [3], Pedro A.f. Galante [2], Garbor Fisher [4], John Gartner [4], Daniela V Frau [3], Roberta Vanni [3], _Paola Caria[3]_, Sabine Mai [1]
University of Manitoba, Cancercare Manitoba Research Institute, Winnipeg-Canada [1] Hospital Sirio-libanes, Centro de Oncologia Molecular, Sao Paulo-Brazil[2] University of Cagliari, Department of Biomedical Sciences, Cagliari-Italy[3] University of Manitoba, Department of Pathology, Winnipeg-Canada[4]

Molecular markers are used as differential diagnostic tools to support patho-logical analysis in thyroid nodules. However, because these markers are also seen in benign thyroid lesions, additional approaches are necessary to differentiate subtypes, tailor specific clinical management and prevent overtreatment. Specifically, this applies to the recently described variant of thyroid cancer called noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP), a variant with an estimated prevalence of 4.4–9.1% of all papillary thyroid carcinomas worldwide. The structural organization of the genome in tumor cells is significantly different from that seen in normal cells and can serve as a structural biomarker to help identify specific thyroid neoplasms. We designed a combined morphological and molecular panel to better determine parameters that can differentiate NIFTP from other papillary thyroid carcinoma (PTC) subtypes. We observed that NIFTP has significantly longer telomeres than classical PTC (CPTC) and follicular variant of papillary thyroid carcinoma (FVPTC). Moreover, super-resolved 3D-structured illumination microscopy demonstrated that NIFTP is a heterogeneous group, and its nuclei present with more densely packed DNA and smaller interchromatin spaces than CPTC and FVPTC, a pattern that resembles normal thyroid tissue. These data are consistent with the observed indolent biological behavior and favorable prognosis associated with NIFTP lesions in which BRAFV600E mutation is absent. In summary, our data suggest that 3D nuclear architecture and 3D-structured illumination microscopy can be useful analytical tools to diagnose and guide the clinical management of NIFTP.

O11 - Plasticity in centromere organization A few megabased sized centromere units can form a holocentromere

Yi-Tzu Kuo [1], Amanda Souza Câmara [2], Veit Schubert [1], Pavel Neumann [3], Jiří Macas [3], Michael Melzer [4], Jianyong Chen [1], Jörg Fuchs [1], Simone Abel [5], Evelyn Klocke [5], Bruno Huettel [6], Axel Himmelbach [2], Dmitri Demidov [1], Frank Dunemann [5], Martin Mascher [2], Takayoshi Ishii [7], andré Marques [8], andreas Houben [1]
Leibniz Institute of Plant Genetics and Crop Plant Research (ipk), Department Breeding Research, Gatersleben-Germany [1] Leibniz Institute of Plant Genetics and Crop Plant Research (ipk), Department Genebank, Gatersleben-Germany [2] Czech Academy of Sciences, Institute of Plant Molecular Biology, Biology Centre, České Budějovice-Czechia [3] Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Department Physiology and Cell Biology, Gatersleben-Germany [4] Julius Kühn-institute (JKI), Institute for Breeding Research on Horticultural Crops, Quedlinburg-Germany [5] Max Planck Institute for Plant Breeding Research, Max Planck Genome-centre Cologne, Cologne-Germany [6] Tottori University, Arid Land Research Center, Tottori-Japan [7] Max Planck Institute for Plant Breeding Research, Department of Chromosome Biology, Cologne-Germany [8]

The centromere is the chromosome region where the kinetochore assembles, and spindle microtubules attach during cell division. In contrast to monocentric chromosomes with one centromere location, holocentric species with a centromere distribution along the entire chromatid exist. We assembled the chromosome-scale reference genome and analyzed the holocentromere and (epi)genome organization of the lilioid Chionographis japonica. Remarkably, each of its holocentric chromatids consists of only 7 to 11 evenly-spaced megabase-sized centromere-specific histone H3-positive units. These units contain satellite arrays of 23 and 28 bp-long monomers capable of

forming palindromic structures. Similar to monocentric species, C. japonica forms distinctly clustered centromeres in chromocenters at interphase. Additionally, the large-scale eu- and heterochromatin arrangement differs between C. japonica and other known holocentric species. Our findings broaden the knowledge about the diversity of centromere organization, showing that holocentricity is not restricted to species with numerous and small centromere units. We demonstrate the unique value of analyzing non-model species for evolutionary comparisons to reveal novelties in even well-studied structures.

O12 Systematic X inactivation studies of sequence resolved balanced X chromosomal rearrangements

Sanam Khan[1], Chelsea Lowther[2], Mana Mehrjouy[1], Malene B. Rasmussen[3], Iben Bache[4], Mads Bak[4], Ryan L. Collins[2], Cynthia C. Morton[5], Michael E. Talkowski[2], Niels Tommerup[1]
University of Copenhagen, Department of Cellular and Molecular Medicine, Copenhagen-Denmark [1] Massachusetts General Hospital, Center for Genomic Medicine, Boston, Ma-United States[2] Department of Healthcare, Danish National Genome Center, Copenhagen-Denmark[3] Copenhagen University Hospital, Rigshospitalet, Department of Clinical Genetics, Copenhagen-Denmark[4] Brigham and Women's Hospital, Department of Obstetrics and Gynecology, Boston, Ma-United States[5]
Danish Cytogenetic Central Registry Study Group*, International Breakpoint Mapping Consortium (IBMC)*, Developmental Genome Anatomy Project (DGAP)*
*Full author list at
https://www.medrxiv.org/content/10.1101/2022.02.15.22270795v1

The majority of balanced X;autosomal translocations (t(X;A)) display 100% skewed X-inactivation due to early embryonic selection against cells with functional partial chromosome X disomy. At cytogenetic resolution only t(X;A) with very telomeric breakpoints are associated with random X-inactivation, likely due to the minimal size of the resulting functional chrX disomy (Schmidt 1991). To explore genomic mechanisms underlying these observations, we have initiated a systematic X-inactivation study of sequence resolved balanced X;autosomal translocations and X-inversions from both affected and healthy carriers based on our sequencing analyses in the International Breakpoint Mapping Consortium and the Developmental Genome Anatomy Project, as well as observations from the literature. So far, our data corroborate the functional disomy hypothesis. A t(X;17)-breakpoint (chrX:154152265-154152265) with a skewed ratio of 88:12, provide an anchor point ~1,5 Mb from the Xq-telomere for addressing tolerance/non-tolerance of functional disomy. In contrast to translocations, balanced intra-chromosomal rearrangements including inversions (inv(X)) should not be subject to functional disomy selection and hence should tolerate random X-inactivation (Tommerup 1993). Our preliminary data partially support this hypothesis but exceptions were observed. We may also uncover locus-specific mechanisms, exemplified by epilepsy and mental retardation restricted to females due to PCDH19 mutation (EFMR)(OMIM 300088). A female inv(X) carrier with EFMR symptoms has an intergenic Xq22 breakpoint 420kb downstream of PCDH19, suggesting a long-range position effect. Males with hemizygous PCDH19 mutations are normal in contrast to heterozygous female carriers. The proposed mechanism underlying EFMR in females is migrational 'cellular interference' due to the presence of a normal and a mutant population during brain development (Depienne 2009). Random X-inactivation in this affected inv(X) carrier would support this hypothesis. Indeed, we predict that female carriers of balanced t(X;A) with breakpoints truncating PCDH19 or its regulatory domain would be phenotypically normal due to a mono-cellular population with 100% skewed X-inactivation.

O13 - A physical map of repetitive elements in the genomes of Iberian Peninsula chiropteran species

Verónica Mestre [1], Francisca Castro [1], Sara Costa [1], Ana Escudeiro [1], Jorge Pereira [2], Paulo Barros [3], Raquel Chaves [1], David Ray [4], João Cabral [3], _Filomena Adega [1]_
University of Trás-os-montes and Alto Douro, Cytogenomics Lab, Vila Real-Portugal [1] University of Trás-os-montes and Alto Douro, Animal and Veterinary Research Centre, Vila Real-Portugal [2] University of Trás-os-montes and Alto Douro, Laboratory of Fluvial and Terrestrial Ecology, Vila Real-Portugal [3] Texas Tech University, Department of Biological Sciences, Lubbock-United States [4]

Despite the huge amount of knowledge currently available on mammalian genomes, most of the research studies are focused on model species, which might not represent the sequence of events underlying the evolution of most mammals. Therefore, it is imperative to conduct research on non-model organisms, such as bats (Order Chiroptera). Bats are the second largest group of mammals with a worldwide distribution both in temperate and tropical regions. In Portugal, is possible to find about 26 species, belonging to four different families. Bats exhibit a high karyotypic and phenotypic diversity compared to other orders of mammals which makes them attractive for evolutionary studies, as well, they had proven to be valuable models to study the repetitive portion of eukaryotic genomes, since they possess a high variability on the physical location of repeats and a distinct repertoire of transposable elements (TEs). TEs dynamism is well known to be the cause of chromosome rearrangements, therefore contributing to genome restructuration. Also, bats

exhibit recent activity of different classes of TEs, suggesting that there may be a connection between these and the evolution of these species' genomes. Physical analysis of TEs and other repeats as ribosomal DNA can thus enlighten our knowledge on its structural and functional impact in genomes as well as to infer phylogenetic relationships among species. Here we present preliminary data on the molecular characterization and physical location of different repeats of bats' genomes from three genera inhabiting the Iberian Peninsula, that are allowing to draw interesting conclusions on the evolutionary path of these elements and of these genomes.

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O14 - The first chicken oocyte nuclear and cytoplasmic whole transcriptomic profile at the lampbrush chromosome stage

Alla Krasikova [1], Tatiana Kulikova [1], Valeria Berngardt [1], Mikhail Schelkunov [2], Nadezhda Makarova [3], Alexei Popov [3], Anna Fedotova [4], Antonina Maslova [1], Anton Fedorov [5]
Saint-Petersburg State University, Laboratory of The Cell Nucleus Structure and Dynamics, Saint-Petersburg-Russia [1] Russian Academy of Sciences, Institute for Information Transmission Problems, Moscow-Russia [2] Skolkovo Institute of Science and Technology, Genomics Core Facility, Moscow-Russia [3] Lomonosov Moscow State University, Belozersky Institute of Physico-Chemical Biology, Moscow-Russia [4] Saint-Petersburg, Laboratory of The Cell Nucleus Structure and Dynamics, Saint-Petersburg-Russia [5]

Hypertranscription during oogenesis is responsible for transformation of the meiotic chromosomes into lampbrush form. However, the spectrum of sequences transcribed on lampbrush chromosomes of birds remained unknown. Here, we systematically characterized chicken oocyte transcriptome at the lampbrush chromosome stage of oogenesis. We performed RNA sequencing of RNA isolated from the cytoplasm and nuclei manually dissected from chicken oocytes at the lampbrush chromosome stage. We examined nuclear and cytoplasmic mRNAs, as well as long and short non-coding RNAs. In chicken ooplasm we detected mature mRNAs and long non-coding RNAs consisting of spliced exons. Visual examination of expression profiles for hundreds of expressed genes revealed that nuclear RNA contains both full-length gene transcripts and intronic sequences. By sequencing small RNA libraries we characterized nuclear and cytoplasmic small house-keeping non-coding RNAs and short regulatory RNAs including more than 150 miRNAs. Since we detected full-length nuclear transcripts for many genes, with reads covering the entire transcription unit, we aimed to detect nascent transcripts in situ. In total, by RNA-FISH we verified transcription of 42 protein-coding genes and 8 long non-coding RNA genes on the lateral loops of chicken lampbrush chromosomes. Among them are genes essential for oocyte maturation and early stages of embryogenesis.
The study was supported by RSF grant #19-74-20075 and was performed using the equipment of Resource Centers 'Molecular and Cell Technologies' (Saint-Petersburg State University) and 'Genomics Core Facility' (Skolkovo Institute of Science and Technology).

Abstracts - Poster presentations

1. Accreditation Quality Control Education

1.P1003 Genome Mapping (optical and electronic) nomenclature and ISCN 2024

Ros Hastings[1], Sarah Moore[2], Jean Mcgowan-Jordan [3]
Nhs Lothian, Genqa, Edinburgh-United Kingdom [1] Sa Genomics Health Alliance, Genetics and Molecular Pathology, Adelaide-Australia[2] University of Ottawa, Cheo Genetics, Ottawa-Canada [3]

The International System for Cytogenomic Nomenclature (ISCN) is used by geneticists globally to describe structural and numerical changes at a genome level. The ISCN Standing Committee recently asked the genetics community to submit proposals for required changes or addendums to ISCN 2020. This presentation will be one of the first opportunities for participants to see the new ISCN 2024, including a new section on genome mapping nomenclature.
Genome mapping technologies (optical, OGM and electronic, EGM) are relatively new cytogenomic techniques that use labelled DNA to assemble genome maps for high-resolution structural variation detection. In addition to the detection of both balanced and unbalanced structural variation, genome mapping can detect copy number changes analogous to chromosomal microarray analysis. Recent publications indicate that genome mapping can replace traditional cytogenomic assays (karyotyping, FISH, and SNP-arrays) in constitutional studies and the evaluation of neoplasia as both balanced and unbalanced structural variants (SVs) can be detected.
The new Genomic Mapping nomenclature (ISCN 2024) for cytogenomics incorporates elements of karyotyping, microarray and region-specific ISCN. This nomenclature will enable scientists and diagnostic laboratories to communicate structural variation, haplotypes and repeat expansion results effectively and without ambiguity to clinicians, public databases and in publications. Participants will gain an understanding of the new cytogenomic mapping nomenclature and see how it compares to array and sequence ISCN nomenclature in different settings through worked examples.
Understanding ISCN and describing the abnormal results accurately can be challenging and this presentation will discuss the tools, webinars and External Quality Assessments (EQAs) available that laboratories can use to train staff and check their competence.

P1032 - Optical Genome Mapping Comparing OGM with other Cytogenomics technologies. Experience on 60 individuals with developmental or fertility disorders.

Doco-Fenzy Martine [1], Pichon Olivier [2], Landais Emilie [3], Moradkhani Kamran [2], Nizon Mathilde [2], Isidor Bertrand [4], Poirsier Celine [3], Mercier Sandra [1], Guichet Agnes [5], Jaillard Sylvie [6], Odent Sylvie [6], Belkayat Aziza [7], Cogne Benjamin [1], Belaud Rotureau Marc Antoine [6], Lambert Latitia [8], Dexheimer Mylene [8], Lebris Yannick [9], Yammine Tony [3], Cogne Benjamin [2], Vasheghani Farahni Faezeh [2], Essid Myriam [7], Sebai Molka [7], Bednarek Nathalie [3], Bonnet Celine [8], Spodenkiewick Marta [3], Stephane Bezieau [1]
CHU Nantes, Institut Du Thorax, Genetic, Nantes-France [1] CHU Nantes, Genetic, Nantes-France [2] CHU Reims, Genetic, Reims-France [3] CHU Nantes, Institut Du Thorax, Genetic, Nantes-France [4] CHU Angers, Genetic, Angers-France [5] CHU Rennes, Genetic, Rennes-France [6] CHU Mongi Slim, Genetic, Tunis-Tunisia [7] CHU Nancy, Genetic, Nancy-France [8] CHU Nantes, Hematology, Nantes-France [9]

The Optical Genome Mapping (OGM) technique is nowadays available with the Bionano protocol. It allows identification of SVs and CNVs in different applications: patients in diagnostic odyssey, with malformative syndromes with or without intellectual disability, or with fertility disorder.
We report results using OGM in constitutional diseases in the genetics department at Nantes University Hospital.
We established OGM technique with 10 known chromosomal abnormalities and we included 60 patients for a solo genome optical mapping using different sample types such as fresh or frozen blood, and even embryonic cell cultures.
Significant structural rearrangements were identified in all known control samples, and in over 50% of the 60 tested samples.
We report a comparison between the Bionano results and different technologies e.g. Karyotype, FISH, aCGH or the genome study illustrated by over 50 cases. Bionano technology was useful in identifiying balanced reciprocal translocation, position of insertions, complex rearrangements, inversion breakpoints, orientation of duplications, deletions in critical regions, complexe breakpoints in known rearrangements and mosaicism. Some of the abnormal results were confirmed by karyotype, FISH, aCGH or genome studies. Some results had been missed by classical algorithms, however OGM succesfully pointed out the abnormal regions in a unique experiment.
We detected interesting CNVs in over 6 other patients including deletions and insertions that linked to the

candidate genes or at the distance in regulatory regions (ex. inversion and FOXG1, deletion near SOX6).
This experience generates practical questions with regard to: resolution of the data compared to sequencing data, interpretation of data analysed due to lack of an internal database, the ISCN formulation of results not yet available, and lack of confirmation techniques or medical guidelines.
Our experience is positive for this complementary approach for etiological diagnosis, for the understanding of genomic structural rearrangements and for genetic counseling.
Grant from Hematology Dpt CHU-Nantes

P1063 - Reciprocal translocations and 3:1 segregation reminder to think of potential effects concerning viability or UPD

Ulrike A. Mau-Holzmann [1], Sylke Singer [1], Ruth J. Falb [1], Guenther Rettenberger [2], Sabine Hoffjan [3]
University of Tuebingen, Department of Medical Genetics and Applied Genomics, Tuebingen-Germany [1] MVZ Neu-Ulm, MVZ Genetikum Gmbh, Neu-Ulm-Germany [2] Ruhr-Universität Bochum, Institut für Humangenetik, Bochum-Germany [3]

Reciprocal translocations are common (about 1 : 1.000).
The translocation heterozygote (carrier) - depending on the type of translocation - has a variable chance to have a healthy child (carrying normal chromosomes or the balanced translocation) or a physically and intellectually abnormal child due to transmission of the chromosomes in an imbalanced manner. Additionally, chromosomal imbalances can result in non-fertilisation or spontaneous abortion.
If certain chromosomes are involved - some translocations need special attention.
Here we present a case where a 3:1 segregation could lead to 2 viable trisomies. The other case is a translocation where trisomic rescue could lead to two different forms of UPD.
These two cases should alert beginners to remember these rare complications of the segregation process in reciprocal translocations.

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2. Animal and Plant Cytogenomics

P1012 - Gametogenesis in hybridogenetic frogs – tracking cellular events of genome elimination and endoreduplication

Mikołaj Kaźmierczak [1], Mikołaj Kaźmierczak [2], Beata Rozenblut-kościsty [1], Krzysztof Kolenda [1], Anna Dudzik [1], Dmitrij Dedukh [3], Pierre-andre Crochet [4], Maria Ogielska [1], *Magdalena Chmielewska [1]*
University of Wrocław, Department of Evolutionary Biology and Conservation of Vertebrates, Wrocław-Poland [1] University of Warsaw, Interdisciplinary Laboratory of Molecular Biology and Biophysics, Warsaw-Poland [2] Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Laboratory of Non-mendelian Evolution, Liběchov-Czechia [3] University Montpellier, Cefe, Cnrs, Univ Montpellier, Ephe, Ird, Montpellier-France [4]

Hybridogenesis is a rare reproductive strategy employed by some interspecies hybrids to produce gametes and propagate to the next generations. Here, we focused on the gametogenesis in two independent European water frogs hybrid complexes from genus Pelophylax: Pelophylax esculentus (containing mixed genomes of P. ridibundus (RR) and P. lessonae (LL)) and P. grafi (containing mixed genomes of P. ridibundus and P. perezi (PP)). Functional gamete production requires removal of one of the parental chromosomal sets, endoreplication of the remaining one and formation of clonal gametes with R or L genome. Cellular processes leading to functional gametes require detailed examination of the earliest stages of gametogenic cells development, i.e. gonocytes. Gonocytes in P. esculentus tadpoles have initially mixed genotypes containing 26 chromosomes (13R, 13L) in diploid hybrids and 39 chromosomes (13R, 26L or 26R, 13L) in triploid hybrids. One of the chromosomal sets (lessonae or ridibundus) undergoes programmed elimination from interphase nucleus by forming micronuclei subsequently degraded by nucleophagy, which happens during gonocytes multiplication in tadpoles and does not occur in adult animals. Apart from the canonical genome elimination and doubling in gonocytes our cytogenetic data reveal unexpected endoreplication of unreduced mixed chromosomal sets in triploid and diploid hybrids, both males and females, leading to formation of polyploid cells. In adult males only 20% of spermatogonial stem cells (descendants of gonocytes) have regular genomic compositions enabling meiosis, while 80% are aneuploid. The opposite proportion is typical for spermatozoa, thus we can conclude that majority of aneuploid cells are eliminated by cell death, leading to partial sterility of adult gonads. In addition, we checked whether the genome elimination mechanism may be evolutionarily conservative in other European hybrid frog P. grafi. We confirm that by detection of micronuclei in the cytoplasm of gonocytes, which serve as a marker of genome elimination in P. esculentus. We also show elimination of P. perezi chromosomes. The gradual genome elimination accompanied by micronuclei formation is an universal process of hybridogenesis in various hybrid lineages of water frogs.

P1021 - Cytogenetics of the hybrid frog Pelophylax grafi and its parental species Pelophylax perezi

Anna Dudzik [1], Dmitrij Dedukh [2], Pierre-André Crochet [3], Beata Rozenblut-Kościsty [1], Hanna Rybka [1], Luká Choleva [4], Maria Ogielska [1], Magdalena Chmielewska [1]
Faculty Of Biological Sciences, University Of Wrocław, Department Of Evolutionary Biology And

Conservation of Vertebrates, Wrocław-Poland [1] Institute of Animal Physiology And Genetics, Czech Academy of Sciences, Laboratory of Non-mendelian Evolution, Liběchov-Czechia [2] Univ Montpellier, Ephe, Ird, Cefe, Cnrs, Montpellier-France [3] Institute of Animal Physiology and Genetics, Czech Academy of Sciences, Laboratory of Fish Genetics, Liběchov-Czechia [4]

The western Palearctic Water Frogs consist of 15 species and four hybrid taxa, which can overcome hybrid sterility. Hybrids reproduce due to hybridogenesis a process that overrides the incompatibility of genomes in the germline cells through the elimination of one of the parental genomes and the following reduplication of the remaining one. This mechanism typically results in haploid clonal gametes. A new generation of hybrid progeny will emerge when the clonal gametes fuse with gametes from the parental species whose genome has been eliminated. Therefore, hybrids live with one of their parental species.
Only one of those hybrids, Pelophylax esculentus (parental genomes: P. ridibundus and P. lessonae), was excessively studied. Unfortunately, studies striving to describe the principles of genome elimination are becoming increasingly difficult due to the overwhelming variety of genetic systems and the frequent existence of triploid individuals. This led us to seek other hybrids forming less complex systems, such as P. grafi (parental genomes: P. ridibundus and P. perezi). Before we start an investigation of mechanisms underlying genome elimination, it is crucial to recognize the chromosomes of both parental species. We found that the karyotype of P. perezi consisted of 2n=26 chromosomes (10 large, 16 small), sharing these basic characteristics with other Pelophylax species. To characterize the P. grafi karyotype and test the integrity of the hybrid's parental sets, we used labelled DNA probes with two expected parental taxa, P. ridibundus and P. perezi. We applied genomic in situ hybridization (GISH) and comparative genomic hybridization (CGH) techniques, as well as AMD-DAPI staining and fluorescent in situ hybridization (FISH) with the RrS1 probe earlier detected in P. ridibundus chromosomes. We have also observed interstitial telomeric sequences in both taxa. Pelophylax perezi chromosomal centromeric regions were less abundant in AT pairs and in the localization of species-specific sequences on P. perezi chromosomes. GISH and CGH confirmed the hybrid nature of P. grafi with chromosomes composed of genomes from two parental species in the F1 state.

P1028 - Drive of chromosomes and programmed chromosome elimination – different sites of the same coin

Andreas Houben [1], Jianyong Chen [1], Alevtina Ruban [1], Anastassia Boudichevskaia [1], Gihwan Kim [1]
Ipk, Br, Gatersleben-Germany [1]

Not necessarily all cells of an organism contain the same genome. Some eukaryotes exhibit dramatic differences between cells of different organs, resulting from programmed elimination of chromosomes or their fragments. Here, we present a detailed analysis of programmed B chromosome elimination in plants. Using goatgrass Aegilops speltoides as a model, we demonstrate that the elimination of B chromosomes is a strictly controlled and highly efficient root-specific process. At the onset of embryo differentiation B chromosomes undergo elimination in proto-root cells. Independent of centromere activity, B chromosomes demonstrate nondisjunction of chromatids and anaphase lagging, leading to micronucleation. Chromatin structure and DNA replication differ between micronuclei and primary nuclei and degradation of micronucleated DNA is the final step of B chromosome elimination. This process might allow root tissues to survive the detrimental expression, or overexpression of B chromosome-located root-specific genes with paralogs located on standard chromosomes. Comparing the cellular process of post-meiotic B chromosome drive in Ae. speltoides and rye with the process of B elimination reveals striking similarities. B chromosomes are often preferentially inherited, deviating from Mendelian segregation. In both processes nondisjunction of Bs occurs despite centromere activity and centromere-tubulin interaction. Spindle symmetry differs between the two processes: during the first pollen mitosis an asymmetric cell division occurs whereas the spindle in roots is symmetrical. As a consequence, in roots, lagging B chromosomes form micronuclei and undergo elimination. In contrast, due to the asymmetric geometry of the spindle at first pollen mitosis, the inclusion of the lagging joint B chromatids into the generative nucleus takes place and chromosome accumulation occurs. Differential RNAseq analysis revealed candidate genes controlling the processes of chromosome drive and chromosome elimination.

P1035 - CAP A satellite DNAs probe mapping on Sapajus cay paraguay and S. macrocephalus by FISH (Platyrrhini Primates)

Simona Ceraulo [1], Aiti Vizzini [1], *Francesca Dumas [1]*
Palermo Univerity, Stebicef, Palermo-Italy [1]

Satellite DNAs have been used as cytogenetic markers for taxonomic and phylogenetic issue in the study of primate genome. Cap-A is a peculiar repetitive sequence found in the genome of same platyrrhini species. The Cap-A sequences probes have been mapped by FISH (fluorescent in situ hybridization) on two Platyrrhini (Primates), Sapajus cay paraguay and S. macrocephalus, in order to study their distribution pattern on chromosomes and to study their potential usefulness as markers. The Cap-A probes have been found on C and CMA3 rich regions of six pairs of chromosomes in both Sapajus species whit an additional pair detected on S. macrocephalus. The

obtained results, have been compared with previous literature data of close phylogenetically New World species. The comparison let to show Cap-A satellite sequences with a genus-specific pattern and slight species-specific pattern that is useful for phylogenetic and taxonomic valuation-

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P1044 - Evolution of gametogenic pathways in reproduction of hybrid males from Pelophylax esculentus complex

Eleonora Pustovalova [1], Dmitrij Dedukh [2], Lukas Choleva [3], Dmytro Shabanov [1]
V. N. Karazin National University, Zoology and Animal Ecology, Kharkiv-Ukraine [1] Institute of Animal Physiology and Genetics, Non-mendelian Evolution, Libechov-European Union [2] University of Ostrava, Faculty of Science, Ostrava-European Union [3]

Hybridization can disrupt conventional gametogenesis, leading to asexual reproduction that allows overcoming hybrid sterility even in the male sex. Premeiotic or meiotic alterations of gametogenesis in asexual organisms prevent pairing between orthologous chromosomes and result in clonal gametes. One of the most intriguing ways of asexual reproduction was found among hybrid water frogs, Pelophylax esculentus. This taxon emerged from interspecific hybridization between the two sexual species, P. ridibundus and P. lessonae. During P. esculentus gametogenesis, one of the parental genomes is selectively eliminated while the other one is endoreplicated and clonally transmitted to haploid gametes. However, the evolution and diversity of such a gametogenic pathway in asexual hybrids are poorly investigated. To analyze the variation of gametogenesis in P. esculentus males, we identified genomes of both parental species in spermatids and spermatocytes of the hybrid males from four localities in Eastern Ukraine using FISH with the species-specific probes. After the examination of cells from the testes of 36 hybrids, we found three gametogenic pathways. In five males, we observed spermatids and spermatocytes with P. lessonae chromosomes and in 12 males, we found spermatids and spermatocytes with P. ridibundus chromosomes. Thus, the genome of one parental species was eliminated premeiotically, and the other was endoreplicated. In 15 males, we found a simultaneous presence of spermatocytes with P. ridibundus chromosomes and spermatocytes with P. lessonae chromosomes only. We suggest that the genomes of either one, another one, or both parental species were eliminated from gonocyte populations. In three males, we observed spermatocytes with an aberrant pairing of P. ridibundus and P. lessonae chromosomes. In 30 males we additionally found aneuploid meiotic metaphases of P. lessonae and P. ridibundus, suggesting that such cells do not form viable gametes. Although hybrids with XY determination system usually suffer from hybrid sterility, water frog males do produce gametes even in diverse ways.

P1098 - Cytogenetic screening of Romanian bovine breeds

Ioana Nicolae [1], Andra Sipos [1], Bianca Groseanu [1], Daniela Vidmichi [1], Adrian Bota [2]
Research@development Institute for Bovine, Cytogenetics, Balotesti-Romania [1] Research@development Station for Buffalo, Reproduction, Sercaia-Romania [2]

Taking into account the role of clinical cytogenentics in the genetic improvements of livestock, the karyotype control of the Romanian cattle and buffalo breeds has been developed, during the last 10 years, in the frame of 6 national projects. The main objective of the chromosomal investigation was to detect the chromosomal abnormalities responsible for the significant degradation of the reproductive activity of the animals at the farm level. A total number of 828 cattle and 287 buffalo, reared in different farms from all over the country, have been karyotyped by using peripheral blood lymphocytes culture. In cattle we identified 113 cases of chromosomal instability and 1 case of leukocyte chimerism XX/XY. The phenotypic effects was expressed by reproductive disturbances (repeated inseminations, lack of oestrus and loss of pregnancy), 4 cases of congenital malformations of the front and rear limbs, a case of foetal abnormality (Schistosomus reflexus), a case of posterior limb malformation and a case of freemartine female. In buffalo, we identified 47 cases of chromosomal instability and Turner's syndrome (2n = 49,X0) in the case of a buffalo females with prominent withers and tight pelvis, all characterized by reproduction disorders. for all animals with chromosomal abnormalities the SCEs test has been used and revealed a very high number of sister chromatid exchanges (SCEs) both in cattle (9-16 SCEs/cell) and buffalo (11-23), with particularly presence of 2-3 interchromatidic exchanges on the same chromosomes. Considering all of this, the high rate of SCEs could be related with the presence of different environmental toxic agents which can induce reproductive disturbances, foetal growth and development disorders of the carriers.

P1125 - A glimpse of the karyotype reshuffling from human to Myotis blythii (Vespertillionidae Chiroptera)

Verónica F. Mestre[1,2,3], Jorge C. Pereira[4], Paulo Barros[3], Raquel Chaves[1,2], David Ray[5], João A. Cabral[3], Filomena Adega[1,2]
[1] CytoGenomics Lab - Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
[2] BioISI - Biosystems & Integrative Sciences Institute, Faculty of Sciences, University of Lisboa, Portugal

[3] Laboratory of Fluvial and Terrestrial Ecology, Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
[4] Animal and Veterinary Research Centre (CECAV), AL 4AnimalS, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal
[5] Department of Biological Sciences, Texas Tech University, Lubbock, TX, USA

Bats (Chiroptera) are a very diverse group of mammals with more than 1400 species. Besides the high number of species, representing about 20% of the mammal species, bats are the second most diverse order within mammals. The family Vespertilionidae is the largest family of the Chiroptera order, with the mouse eared bats (Myotis) exhibiting a very conserved karyotype, with 2n=44 chromosomes, with only a few exceptions.
Zoo-FISH methodologies allows the comparison of genomes of very different species, providing hints on the possible karyotype rearrangements occurred throughout the evolutionary process of speciation. In this work we present the comparative chromosome map of Myotis blythii using human painting probes. The comparison of this map with the already existent from Myotis myotis allowed to disclose the extent and type of reshufflings occurred between these Myotis species and between these and human. Besides shedding light on the evolutionary events that are shaping the extant genomes of this group, this analysis will also provide the genome coordinates of the evolutionary breakpoints that preceded the rearrangements observed, and consequently, the sequences lying on those regions, that most probably contributed and are, most probably, contributing to the karyotype evolution of these diverse group.
This work was supported by the projects UIDB/CVT/00772/2020, UIDB/04033/2020, UID/MULTI/04046/2021 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT) and the PhD grant with the reference 2021.07537.BD.

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3. Clinical Cytogenomics

P1002 - A very rare double chromosome 9 mosaicism a case report

Andreja Zagorac [1], Mirjana Miksić [2], Iva Opalič [1], Boris Zagradi nik [1], Bojan Korpar [2], Nadja Kokalj Vokač [1]
University Medical Centre Maribor, Clinical Institute for Genetic Diagnostics, Maribor-Slovenia [1] University Medical Centre Maribor, Department of Perinatology, Maribor-Slovenia [2]

Introduction: Occurrence of three cell line mosaicism with chromosomal abnormalities of an autosomal chromosome in the same individual is a very rare condition. We report on a one-year-old girl presented with ventriculomegaly, intraventricular hemorrhage, a slightly thinner corpus callosum, neurological symptomatology, feeding disturbances, hypotonia, congenital heart anomalies, ovarian cysts, minor abnormalities of the palms and feet, and dysmorphic features: asymmetrical palpebral fissures, hypertelorism, short philtrum, dysmorphic low set ears, high palate and retrognathia.
The girl has a mosaic karyotype including full trisomy 9, normal cells and in a majority of the cells trisomy of 9p.

Materials and Methods: aCGH analysis using 4x180K array, karyotyping, FISH analysis with DNA probes along the chromosome 9 and QF-PCR using chromosome 9 specific microsatellite markers on peripheral blood, were performed.
Results: Array CGH revealed a gain of the whole short arm of chromosome 9 in approximately 80% of cells and a gain of the whole long arm of chromosome 9 in approximately 20% of cells: arr(9p)x3[0.8],(9q)x3[0.2]. Metaphase analysis demonstrated mosaic karyotype: 47,XX,+del(9)(q13)[65]/46,XX[35]. FISH and QF-PCR confirmed/identified the third cell line with trisomy of chromosome 9. The karyotype was re-written:
47,XX,+del(9)(q13)[60]/47,XX,+9[20]/46,XX[20]
Conclusion: Our data demonstrate that the presented girl is a mosaic with three cell lines. Trisomy 9p dominated in 60%, trisomy 9 and normal cell line were observed in approximately 20% each. The exact contribution of each chromosomal abnormality on the girl's phenotype could not be determined because there are similarities between individuals with trisomy 9 mosaicism and those with duplications of 9p. Although aCGH should be the first-tier test for clinical diagnosis of chromosome abnormalities detection of mosaicism requires a multistep diagnosis approach.

P1007 - the finnish national collection of balanced translocations and inversions facilitates gene mapping

Tiia Luukkonen [1], Minna Pöyhönen [2], Aarno Palotie [3], _Teppo Varilo [4]_

Fimm Institute for Molecular Medicine Finland, National Institute for Health and Welfare, Helsinki-Finland [1] Dept. of Clinical Genetics, Helsinki University Central Hospital, Dept. of Medical and Clinical Genetics, Helsinki-Finland [2] Broad Institute of Harvard and Mit, Fimm Institute for Molecular Medicine Finland, Helsinki-Finland [3] Dept. of Medical and Clinical Genetics, National Institute for Health and Welfare, Helsinki-Finland [4]

We have gathered a nationwide collection of all carriers of reciprocal balanced translocations and inversions from every Medical Genetics Department and Clinical Genetics Laboratory in Finland. Our database contains medical records of carriers and their

relatives carrying the same rearrangement identical by descent as well as other relatives for comparison. To date, we have systematically assembled 3016 carriers and their relatives, and gathered samples (n=124; DNA, RNA and cells from each). We are in process of collecting more biological samples, and linking our data to the comprehensive national disease registers. Additionally, we collaborate with the International Breakpoint Mapping Consortium (Tommerup N et al. 2015 Cancer Genetics).

We utilize this valuable collection as a gene mapping tool. We have searched distinct diseases or traits segregating with a given chromosomal break in the families, and identified their precise molecular location on DNA level. We focus on families, which appear to have a striking correlation of a balanced translocation or an inversion and an abnormal phenotype. In individuals from each of these families, we have performed standard cytogenetic analyses, copy number analyses (genotyping and aCGH), genome-wide paired-end sequencing, and capillary sequencing with an objective to identify specific breakpoints for each translocation and to rule out other causative genetic factors. In our first gene-mapping pilot, we identified a potential positional candidate gene for intracranial and aortic aneurysm (Luukkonen T et al. 2012 JMG). The second revealed a familial translocation t(1;12)(q43;q21.1), which truncates a gene in a family suffering from vascular phenotypes and strokes (Luukkonen T et al. 2018 Mol. Genet. Genomic Med.). The current one is a translocation t(2;18), which associates dyspraxia families with a most interesting gene; there is a University of Copenhagen requirement to perform cellular studies prior to making it public.

Our results demonstrate the feasibility of genome-wide paired-end sequencing for the characterization of balanced rearrangements and identification of candidate genes in patients with potentially disease-associated chromosome rearrangements. This unique registry will be of benefit to both researchers and clinicians by facilitating diagnostic purposes, genetic counseling, and subsequent follow-up.

P1008 - Genetic hearing loss screening by MLPA in a cohort of Portuguese patients

Cristina Candeias [1], Manuela Mota Freitas [1], Carolina Alves [2], Cláudia Falcão Reis [3], Natália Oliva-teles [4]
Cgmjm/chudsa; Umib/icbas, Itr, Genetics, Porto-Portugal [1] Utad; Cgmjm/chudsa, Genetics and Biotechnology, Vila Real-Portugal [2] Cgmjm/chudsa; Umib/icbas, Itr; Icvs/3b’s, Genetics, Porto-Portugal [3] Cgmjm/chudsa; Umib/icbas, Itr; Medcids, Genetics, Porto-Portugal [4]

Introduction: Hearing loss (HL) is the most common sensory impairment in humans, affecting around 466 million people worldwide. Genetic HL occurs mostly in non-syndromic forms, although several syndromes are associated with its emergence. Hundreds of genes involved in HL have been described and many can cause dominant and/or recessive forms. Given its high prevalence and the impairment in patients’ lives, HL is a condition that benefits from early detection and molecular diagnosis, allowing a more targeted clinical intervention, follow-up and treatment.

Material and methods: Between January 2021 and December 2022, 74 blood samples (56 probands and 18 relatives) were analysed using the SALSA® MLPA® Probemix P163-E1 GJB-WFS1-POU3F4 panel.

Results: of the 56 analysed probands (20 females and 36 males), 11 (19.6%) showed genetic anomalies: nine c.35delG mutation in exon 2 of the GJB2 gene (five homozygous, two heterozygous and two in a compound heterozygosity with a deletion of the GJB6 and CRYL1 genes), one mutated GJB2 allele with the c.101T>C variant in exon 2 of the gene and one deletion c.167delT ex2 GJB2, both in heterozygous presentation.

Discussion: It was possible to establish a genetic aetiology for the onset of the condition in seven of our patients. c.35delG mutation in exon 2 of the GJB2 gene in homozygosity was the most frequent alteration detected in our study, which is in agreement with the literature since this is the most frequently reported variant in cases of autosomal recessive HL worldwide. Thus, MLPA panel P163-E1 showed to be a very valuable, cost-effective first tier technique to screen frequent variants associated with HL. This work contributed to a better characterization of the Portuguese hearing-impaired population, regarding the genetic causes of HL, with positive impact in clinical decision-making and genetic counselling.

P1009 - Chromosomal abnormalities in donor gamete candidates in a Public Bank retrospective analysis 2013-2022

Manuela Mota Freitas [1], Cristina Candeias [1], Elisa Lopes [2], Isaltina França [2], Paula Oliveira [2], Sílvia Pires [2], Natália Oliva-telles [3]
Cgm-chudsa, Umib-icbas, Itr, Genetics, Porto-Portugal [1] Cgm-chudsa, Genetics, Porto-Portugal [2] Cgm-chudsa, Umib-icbas, Itr, Medcids, Genetics, Porto-Portugal [3]

Introduction: In Portugal, the Public Gamete Bank is a service provided by the National Health Service (NHS), which is responsible for the selection of gamete donors. The collection of oocytes and sperm is carried out in specialized Centers, located in NHS Public Hospitals. Gamete donors are selected among phenotypically normal young men and women, on a voluntary basis. In the first medical genetics consultation, after consent, the candidates are invited to do specific genetic tests, such as a karyotype, in order to exclude chromosomal abnormalities in descendants.

Material and methods:

Between 2013-2022, the karyotypes of 525 donors (383 women and 142 men), with ages between 18 and 41, with an average age of 27 years, were analyzed in the Cytogenetics Unit of Centro de Genética Médica Jacinto de Magalhães/ CHUdSA, Porto, Portugal.
The chromosome analysis was performed on GTL banded metaphases obtained for peripheral blood cultures according to standard methods.
Results:
of the total of 525 individuals, 10 (1,9%) cases showed chromosomal alterations: four with sex chromosome aneuploidy mosaicism; three translocations: t(4;17), t(11;22) and t(1;14); two karyotypes with a mosaic marker chromosome and one translocation t(9;22) mosaicism, suggestive of an acquired chromosomal anomaly. Two other cases revealed chromosomal instability.
Discussion: The authors compare the results obtained in the individuals in the present study with literature reports and indicate the relatively high percentage of chromosomal alterations in a phenotypically normal healthy population, highlighting the importance of performing specific genetic studies for adequate and sometimes urgent genetic counseling.

P1010 - CLASSICAL GENETIC TECHNIQUES ARE STILL IN USE a case with low mosaicism

Esther Cuatrecasas [1], Maria Lluïsa Ramírez [1], Teresa Zabala [1], Carlos Belbel [1], Rosa Diop [1], Didac Casas [2], Lourdes Vega-hanna [3], Jordi Genoves [4], Daniel Castillo [4], Loreto Martorell [4], Sandra andreu [1]
Hospital Sant Joan De Deu, Citogenètica, Esplugues Del Llobregat-Spain [1] Hospital Sant Joan De Deu, Genetics, Esplugues Del Llobregat-Spain [2] Hospital De La Santa Creu I Sant Pau, Genetics, Barcelona-Spain [3] Hospital Sant Joan De Deu, Genètica Molecular, Esplugues Del Llobregat-Spain [4]

Introduction
Low grade mosaicism is still a problem in genetic diagnosis. While one is working hard to solve this problem with new molecular techniques, the use of classical genetic methods remains a good cost-effective solution.
We present a new case where the use of classical techniques was essential to resolve the patient's phenotype.
Case description
An 11-month-old girl visited the Ophthalmology Service of our Hospital due to a bilateral coloboma. CGH-Array and NGS studies were requested. No SNV variants or CNV changes were detected in relation to the observed pathology. A year later, an NGS reanalysis was performed, with the same result.
When the girl was three years old, a paediatrician asked for a karyotype, because of the strong suspicion of cat's eye syndrome (CES).
Results
The karyotype showed low mosaicism with a marker chromosome in 12% of the counted metaphases (47,XX,+mar[6]/46,XX[44]). Result of FISH studies with the probes for DiGeorge/VCFS TUPLE1 and 22q13.3 (CYTOCELL)were inconclusive.
As the clinical suspicion remained strong, MLPA technique was carried out; mosaicism of about 13% was detected for duplication of the 22q11 region
Thus the suspicion of mosaic CES was confirmed with the karyotype and MLPA studies.
To work toward the implementation of the Digital PCR technique, this patient was analysed, confirming the results obtained.
Conclusions discussion
Although new technologies provide more precise information, one of their limitations is the detection of low mosaicism. In case of suspicion of a genetic syndrome that is possibly occurring with low mosaicism, we strongly recommend using classical techniques such as karyotype, FISH or MLPA. It is important to note that every technique has limitations, and one of the important tasks of the geneticist is to choose the right technique to achieve the most accurate result.

P1016 - CACNA1B GENE AND AUTISM CORRELATION A CASE REPORT

Silvia Motta [1], Dario De Vita [1], Luciana Di Schiena [1], Martina Rimoldi [1], Lucia Tararà [1], Fulvio Ferrara [1], Lucy Costantino [1], Sabine Stioui [1]
Centro Diagnostico Italiano – Cdi Spa, Department of Molecular Genetics and Cytogenetics, Milan-Italy [1]

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by three core behavioral symptoms: impairment in social interaction skills, impairment in communication, and repetitive and restrictive behaviors and interests. Prevalence of ASD vary and is estimated to be globally around 0.06–1.4%. The exact etiology of ASD is unknown, but it has been suggested to be multifactorial and clinically heterogeneous.
A recent systematical review of existing publications reveals a role of voltage-gated calcium channels (VGCCs) genetic variants in the pathogenesis of ASD, making it a promising therapeutic target.
Furthermore in the literature the presence of CNVs, in the CACNAB1 gene encoding for a pore protein-forming subunit of VGCC that controls neurotransmitter release from neurons have been described associated with ASD.
Despite this evidence up to date only 5 cases with isolated duplications of gene CACNA1B with benign classification have been reported.
In our study we report the result about the analysis of a 14-year-old male patient with ASD phenotype in which a 261 Kb duplication at 9q34.3 band involving CACNA1B gene (classified as VUS) was identified by CGH array. To clarify the involvement of this duplication in the pathology occurrence, the analysis was extended to the parents and a maternal origin of the duplication was revealed.

The presence of this CNV in the healthy mother supports a probable benign classification of the CACNA1B gene duplication. The extension of the analysis to the parents was relevant to clarify the interpretation of the CNV. Other further DNA sequence analysis could better elucidate the mechanism underline the disease.

P1017 - Population screening for 15q11 q13 duplications corroboration of the difference in impact between maternally and paternally inherited alleles

Ilse Parijs [1], Nathalie Brison [1], Leen Vancoillie [1], Machteld Baetens [2], Bettina Blaumeiser [3], Sébastien Boulanger [4], Julie Désir [4], Boyan Dimitrov [5], Nathalie Fieremans [5], Katrien Janssens [3], Sandra Janssens [2], Axel Marichal [4], Björn Menten [2], Colombine Meunier [4], Kim Van Berkel [5], An Van Den Bogaert [5], Koenraad Devriendt [1], Kris Van Den Bogaert [1], Joris Robert Vermeesch [1]
University Hospital Leuven, Center for Human Genetics, Leuven-Belgium [1] University Hospital Ghent, Center of Medical Genetics, Ghent-Belgium [2] University Hospital Antwerp, Center of Medical Genetics, Antwerp-Belgium [3] Institut De Pathologie Et De Génétique Gosselies, Center for Medical Genetics, Charleroi-Belgium [4] University Hospital Brussels, Center for Medical Genetics, Brussels-Belgium [5]

Maternally inherited 15q11-q13 duplications are generally found to cause more severe neuro-developmental anomalies compared to paternally inherited duplications. However, this assessment is mainly inferred from the study of patient populations, causing an ascertainment bias. Here, we analyze the low coverage genome-wide cell-free DNA sequencing data obtained from pregnant women during non-invasive prenatal screening (NIPS). We detect 23 15q11-q13 duplications in 333,187 pregnant women (0.0069%), with an approximately equal distribution between maternal and paternal duplications. Maternally inherited duplications are always associated with a clinical phenotype (ranging from mild learning difficulties to intellectual impairment, epilepsy and psychiatric disorders), while paternal duplications are associated with milder phenotypes (from normal to learning difficulties and dyslexia). This data corroborates the difference in impact between paternally and maternally inherited 15q11-q13 duplications, contributing to the improvement of genetic counselling. We recommend reporting 15q11-q13 duplications identified during genome-wide NIPS with appropriate genetic counselling for these pregnant women in the interest of both mothers and future children.

P1026 - Balanced complex chromosomal rearrangement of chromosome 2 in an infertile male

Tea Mladenić [1], Thomas Liehr [2], Anita Bari ić [1], Niklas Padutsch [2], Stefanie Kankel [2], Antun Gr ković [3], Nada Starčević Čizmarević [1], Jadranka Vraneković [1]
Faculty of Medicine, Medial Biology and Genetics, Rijeka-Croatia [1] Jena University Hospital, Friedrich Schiller University, Institute of Human Genetics, Jena-Germany [2] University Hospital Rijeka, Urology, Rijeka-Croatia [3]

Complex chromosomal rearrangements (CCRs) are rare structural chromosomal aberrations characterized by many breaks in single or multiple chromosomes. While almost all unbalanced CCRs cause an abnormal phenotype, balanced CCRs may remain undetected until reproductive age. There are several factors that are thought to influence the fertility of carriers of a CCR, such as the number and position of breakpoints involved in the rearrangement, the relative size of the derivative chromosomes, and the presence or absence of recombination within the paired segments of the rearrangement.
We report an azoospermic patient with primary hypogonadism who has a balanced CCR with four breakpoints and a pericentric inversion in chromosome 2.
A 48-year-old Caucasian male was referred for chromosomal and Y-chromosome microdeletions analysis for infertility due to primary hypogonadism and non-obstructive azoospermia. The test result for Y-chromosome microdeletions was negative. Cytogenetic analysis revealed a derivative chromosome 2. To further characterize the exact chromosomal breakpoints, a high-resolution fluorescence in situ hybridization (FISH) was performed, using a multicolor banding (MCB) probe set for chromosome 2. FISH analysis revealed a final karyotype as 46,XY,der(2)(pter->p16.1::q21.1->q36.3::p16.1->p15::q21.1->p15::q36.3-> qter).
This report demonstrates a unique male karyotype with a balanced CCR of chromosome 2 in an azoospermic patient. Spermatogenic arrest can be influenced by both aberrant segregation of the rearranged chromosome and chromosome breakpoints that disrupt some autosomal genes potentially involved in spermatogenesis of the carrier. As a result of the proposed mechanisms, a high frequency of chromosomally abnormal spermatozoa is produced. Therefore, genetic counseling should be offered to a carrier of this exceptional CCR, but a successful outcome of assisted reproduction is highly unlikely.

P1030 - Chromosomal abnormalities in male partners of infertile couples

Elisavet Kouvidi [1], Haroula Tsarouha [1], Christina Katsidi [1], Sophia Zachaki [1], Amelia Pantou [1], Hara Tsimela [1], Emmanouel Kanavakis [1], Ariadni Mavrou [1]
Genesis Genoma Lab, Genetic Diagnosis, Clinical Genetics & Research, Cytogenetics, Athens-Greece [1]

Background
Infertility in a couple may be caused by problems in one or both partners. The frequency of chromosomal abnormalities in infertile couples is higher than in the general population (0.7%), varying from 1.3-15.0%.
Aim
To determine the prevalence of chromosomal abnormalities in male partners of infertile couples undergoing assisted reproduction techniques (ART).
Materials and Methods
Cytogenetic analysis was performed on peripheral blood lymphocytes using standard G-banding techniques in 2.159 men from infertile couples referred to Genesis Athens Clinic between June 2016 and December 2022. The mean age of the individuals studied was 40.02 years. In all cases, the karyotypes of their female partners were normal (46,XX). At least 25 metaphases were analyzed and in cases of suspected mosaicism, 100 cells were counted. Chromosomal polymorphisms such as prominent satellites, increased heterochromatic regions and pericentric inversions of chromosome 9 were not included.
Results
2.115 men (98%) had a normal karyotype, while in 44 (2%) a chromosomal rearrangement was observed. 27 men (1.25%) had autosomal chromosomal abnormalities (16 reciprocal translocations, 6 Robertsonian translocations and 5 inversions). In 15 cases (0.7%), sex chromosome abnormalities were detected (5 Klinefelter syndrome, 5 mosaics Klinefelter, 2 sex reversal syndrome (46,XX), 2 mosaics 45,X/46,XY, and 1, 47,XYY syndrome). Two men (0.06%) were found to carry a supernumerary marker chromosome (47,XY,+mar).
Conclusions
The study confirms the increased frequency of chromosomal rearrangements in male partners of infertile couples and highlights the significance of chromosomal analysis in infertile couples in advance of ART treatment. Cytogenetic findings should be taken into consideration and genetic counselling should be offered to the couple in order to understand the problem and select, with the aid of their physician, the appropriate protocol, including the possibility of preimplantation genetic screening.

P1037 - Different strategies for the detection of copy number variations from exome sequencing data

Vladimíra Vallová [1], Kristína Handzušová [1], Markéta Wayhelová [2], Petr Brož [1], Aneta Mikulá ová [3], Jan Smetana [1], Renata Gaillyová [4], Petr Kuglík [1]
Faculty of Science, Masaryk University, Department of Experimental Biology, Brno-Czechia [1] University Hospital Brno, Centre of Molecular Biology and Genetics, Brno-Czechia [2] Newcastle University, Biosciences Institute, Newcastle Upon Tyne-United Kingdom [3] University Hospital Brno, Department of Medical Genetics and Genomics, Brno-Czechia [4]

The development of special algorithms has recently brought copy-number variation (CNV) detection by exome sequencing (ES) much more to the forefront. There is no one-size-fits-all approach for reliable detection of CNVs from ES data. On the contrary, many different approaches combining capture kits and bioinformatics approaches are being tested for their detection.
Total of fifteen samples with twenty rare CNVs (14,5 kb – 8 Mb) - using high-resolution chromosomal microarray analysis (CMA) as a standard - were selected for comparison of two different capture designs and five different read-depth based CNV calling strategies: Human Core Exome (HCE) from Twist Biosciences (CNVRobot (CNVR), in-house pipeline (IHP)) and SureSelect All Exon v7 (SSEL) from Agilent Technologies (Circular Binary Segmen_tation (CBS), Hidden Markov Model (HMM), ExomeDepth).
of the twenty rare CNVs tested, three strategies (CNVR and IHP for HCE and ExomeDepth for SSEL) were able to identify all of them. The CBS- and HMM-based strategies (SSEL) missed three and two CNVs, respectively.
Differences were observed between the size of CNVs obtained by CMA and by the different ES CNV calling strategies. These differences arise from the different CMA probes and ES targets distributions along with the variable bioinformatics pipeline settings in the case of ES. In addition, ES CNV calling was able to detect intra-exonic rearrangements (in ZC4H2, GRIN2A, BRCA1, RNF125 genes), confirmed by qPCR.
In summary, ES is a suitable approach for CNV detection. However, its reliability strongly depends on sequencing quality and data uniformity. In general, the combination of different CNV calling strategies can improve the reliability of CNV detection from ES data.
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P1041 - A case report of the interstitial duplication the short arm of chromosome 7

Valentyna Kurakova [1], Vira Galagan [1], Olena Baronova [1], Diana Mykhailova [1], Marina Tsygankova [1], Olga Zhurakhovska [1], Victoria Cherevashko [1]
National Specialized Children Hospital “okhmatdyt”, The Center of Medical Genetics, Kyiv-Ukraine [1]

Introduction. Partial interstitial duplications are very rare de novo chromosomal pathology. The frequency is less than 1/1,000,000. According the literature 7p22.1 microduplication syndrome is clinically significant, which characterized by intellectual disability, psychomotor and speech delay, craniofacial dysmorphism, and cryptorchidism. Cardiac (eg, patent foramen ovale and atrial septal defect), as well as renal, skeletal, and eye abnormalities may also be associated.
Materials and methods. Clinical, standard karyotyping, Fluorescence in situ hybridization (FISH) using TelVysion 7p, Vysis, Chromosome microarray (CMA).
Discussion. A child from the first pregnancy, the first birth at 38 weeks. Birth weight 3600, height 54 cm, Apgar score 6-7. At the center of medical genetics the child was consulted for the first time at the age of 1.5 months.
Phenotype: Prominent frontal bumps, oblique eye fissure, hooked nose, partial choanal atresia on the right, drooping corners of the mouth, microgenia, preauricular fistulas, pinna deformity, thickened earlobes with notches, hypertelorism, and hypoplasia of the nipples. Contractures of the hand and knee joints, deep palmar and plantar folds, hypogonadism, including cryptorchidism.
Magnetic resonance imaging of the brain - minimal hypoplasia of the posterior parts of the corpus callosum, hypoplasia of the olfactory bulbs.
Standard chromosomal analysis showed, that there is an additional material of unknown origin on the short arm of the chromosome 7. FISH-analysis showed the two copies of signal 7ptel on the both short arms of the chromosome 7, which suggested a possibility of duplication or insertion in the analysed region. The karyotype of the proband: 46,XY,der(7)add(7)(p21), dn.ish 7(VJIyRM2185,VIJyRM2000)x2. The parent's karyotypes were normal.
CMA was performed on the Afymetrix platform using Optima CytoScan Array chips. It showed the partial duplication of the short arm of the chromosome 7. The size of duplicated region 12 Mb and contains 95 genes.
Total result:
arr[GRCh38]7p22.3p21.2(1,808,520_14,091,126)x3
The diagnosis was verified when the child was 3 months old. Further monitoring of the child's development by a multidisciplinary group of specialists is recommended.
Conclusion. Unbalanced chromosomal rearrangements often require the use of different laboratory methods and different levels of resolution to understand the mechanisms of rearrangement formation.

P1043 - First case report of a patient with three copies of distal 16p12.1p11.2 (BP1 BP3 region) and four copies of proximal 16p11.2 (BP4 BP5 region) inherited from both parents

Leona Morožin Pohovski [1], Ivona Sansović [1], Katarina Vulin [1], Ljubica Odak [1]
Children's Hospital Zagreb, Scientific Centre of Excellence for Reproductive and Regenerative Medicine (cerrm), University of Zagreb School of Medicin, Department of Medical Genetics and Reproductive Health, Zagreb-Croatia [1]

Recurrent proximal copy number variants (CNVs) at 16p11.2 are among the most frequent genetic causes of neurodevelopmental disorders (NDDs). Deletions and duplications at 16p11.2 are also associated with increased incidence of structural brain anomalies. The phenotype for distal 16p12.1p11.2 trisomy and proximal 16p11.2 tetrasomy is unknown. We present a three-year-old boy with microcephaly, trigonocephaly, dysmorphic features; round face, prominent ears, epicanthal folds, motor delay and autism spectrum disorder. The genetic investigation was performed on DNA from the patient and both parents using Agilent 60K oligonucleotide array-based comparative genomic hybridization (SurePrint G3 Unrestricted CGH ISCA v2). The data was analysed and interpreted using Agilent CytoGenomics software. Microarray analysis showed a single copy gain of a 1.187 kb segment in the 16p12.1p11.2 region and a two copy gain of a 525 kb segment in the 16p11.2 region. Parental analysis revealed a 1.7. Mb duplication at 16p12.1p11.2 (BP1-BP5 region) in the father while the mother had a 525 kb duplication in the 16p11.2 region (BP4-BP5). The patient inherited the whole abnormality from each parent, resulting in partial trisomy 16p12.1p11.2 and partial tetrasomy 16p11.2 (double duplication). Double duplication are very rare chromosomal rearrangements. To our knowledge, this is the first patient described in the literature who inherited 16p11.2 duplications from both parents. With this case report, we want to contribute to the phenotype-genotype correlation in patients with partial trisomy 16p12.1p11.2 (BP1-BP3 region) and partial tetrasomy 16p11.2 (BP4-BP5 region).
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P1046 - Cytogenetic analysis of induced pluripotent stem cell (iPSC) cultures derived from dermal fibroblasts

Nikoletta Selenti [1], Anastasios Kollias [2], Chrystalena Sofokleous [1], Florentia Papastefanaki [3], Rebecca Matsas [2], Joanne Traeger Synodinos [1]
Medical School, National and Kapodistrian University of Athens, “aghia Sophia” Children’s Hospital, Laboratory of Medical Genetics, Athens-Greece [1] Hellenic Pasteur Institute, Laboratory of Cellular and Molecular Neurobiology-stem Cells, Athens-Greece [2] Hellenic Pasteur Institute, Laboratory of Cellular and Molecular Neurobiology-stem Cells, Human Embryonic and Induced Pluripotent Stem Cell Unit, Athens-Greece [3]

Introduction:
Induced pluripotent stem cells (iPSCs) are generated by cellular reprogramming of differentiated cells. Resembling embryonic stem cells, iPSCs retain the capacity for self-renewal and can be differentiated into derivatives of all three germ layers, providing an ideal tool to model human development and diseases in vitro and a drug-screening platform in a human context. Among limitations, chromosomal instability has been associated with prolonged in vitro maintenance and expansion of iPSCs.
Material and Methods: Skin fibroblasts from male and female donors (38-51 years) were reprogrammed to iPSCs, according to established protocols (doi: 10.1016/j.cell.2007.11.019) and were maintained in culture either on feeders, or under feeder-free conditions. Conventional karyotyping analysis of 15 samples was performed between passages 20-45.
Results: Chromosomal abnormalities were detected in four samples as follows: 47,XY,+3; 47,XY,+17; 46,XY,inv(X)(p11.3q12); and 47,XX,+20,der(1), add(1)(q25). Re-examination of affected clones at earlier passages revealed that the first three were free of chromosomal abnormalities, whereas the last was already affected, indicating that this chromosomal alteration conferred privilege to the carrier cells that dominated the culture over passages.
Conclusion: Our data, in line with previously reported genomic instability of iPSCs, show that prolonged culturing and replace-by-passaging methods are possible factors contributing to chromosomal changes. Yet, some chromosomal abnormalities were detected even at earlier passages. Therefore, systematic karyotypic analysis is justifiably recommended as a necessary step of the iPSC quality control pipeline.

P1048 - Diagnosis in Emanuel syndrome a challenge for geneticists

Eusebiu Vlad Gorduza [1], Setalia Popa [1], Violeta Martiniuc [2], Irina Resmerita [1], Mihaela Gramescu [1], andreea Florea [2], Ana Maria Grigore [2], Claudia Banescu [3], Alina Boglis [3], George andrei Crauciuc [3]
”grigore T. Popa” University of Medicine and Pharmacy, Medicine of Mother and Children, Iasi-Romania [1] ”cuza Voda” Obstetric and Gynecology Hospital, Medical Genetics, Iasi-Romania [2] George Emil Palade University of Medicine, Pharmacy, Science and Technology, Center for Advanced Medical and Pharmaceutical Research, Targu Mures-Romania [3]
Emanuel syndrome is a rare chromosomal disorder (about 400 reported cases). The syndrome is diagnosed neonatally, the main diagnostic elements being growth retardation, facial dysmorphia and congenital visceral anomalies. Because of its rarity and variable phenotypic appearance, clinical diagnosis is often difficult. We present two cases of ES diagnosed in different ways. Case 1. At birth, intrauterine growth retardation, facial dysmorphia, genital and hand abnormalities, atrial septal defect, muscle hypotonia and breathing difficulties were discovered. Due to the neurological symptoms, an NGS test for neurodevelopmental disorders was performed which indicated the existence of three copies of genes located on chromosome 11: CBL and KMT2A. Next, karyotype (47,XY,+der(22)t(11;22)(q23;q11)) and array-CGH (arr[GRCh37] 11q23.3 q25(116722290_134937416)x3, 22q11.1q11.21 (168 88900_20312661)x3) were performed, both confirming the diagnosis of ES. Case 2. At birth, the child presented: intrauterine growth retardation, cleft lip and palate, atrial septal defect and bilateral cryptorchidism. The first clinical examination was done at 1 and a half year and showed muscle hypotonia, global developmental delay, craniofacial dysmorphism with microcephaly and microretrognathism, and congenital heart anomaly. The karyotype showed the presence of a marker chromosome suspected to be a derivative of chromosome 22 (47,XY,+mar). To validate this hypothesis, MLPA was applied with the P064 kit that attests 22q11.1q11.23 microduplication. Subsequently, kits P036, P070 and P286 were applied that confirmed that the marker chromosome contained microduplications of both chromosome 22 and chromosome 11 regions. In both cases, the karyotype of the parents was normal, the anomalies identified in the children being considered de novo. The two cases presented by us indicate the difficulties of clinical diagnosis in ES and attest the need to use various cytogenetic and cytogenomic methods to confirm the diagnosis of the disease.

P1049 - familial case of X;9 translocation

Natalia Oparina [1], Olga Solovova [2], Yulia Kotalevskaya [3], Olga Schagina [4], Vyacheslav Chernykh [4]
Moscow Regional Scientific Research Clinical Institute, Petrovsky National Research Centre of Surgery Rams, Medical Genetic, Moscow-Russia [1] Moscow Regional Scientific Research Clinical Institute,research Centre for Medical Genetics, Medical Genetic, Moscow-Russia [2] Moscow Regional Scientific Research Clinical Institute, Medical

Genetic, Moscow-Russia [3] Research Centre for Medical Genetics, Medical Genetic, Moscow-Russia [4]

Background: Only a few familial cases of X-autosome translocations have been reported as they are often associated with meiotic defects and infertility. The phenotype of a carrier may also depend on whether the X-inactivation spreads on to the translocated autosomal segment.
Clinical case: We report on a family with an X;9 translocation in a mother and her daughter. The proband is a newborn girl with congenital malformations (ventricular septal defect, renal hypoplasia) and following phenotypic features: dolichocephaly, bird-like face, orbital hypertelorism, micrognathia, low set ears, gothic palate, narrow shoulders, extra ribs, limited hip abduction.
The proband's mother (34 y) is a healthy individual with normal intelligence. She had three natural pregnancies (one childbirth and two spontaneous abortions); the abortion material was not examined.
Material and methods: Chromosome analysis using GTG-staining and FISH with subtel XY DNA probes was performed on cultured lymphocytes. The X chromosome inactivation (XCI) was evaluated by methylation assay of the CAGn polymorphic locus of AR/HUMARA gene.
Results: Proband's karyotype was defined as 46,X,der(X)t(X;9)(q28;q22)dmat, i.e. unbalanced with a deletion of Xq28-qter and a duplication of 9q22-qter. The mother had a balanced translocation: 46,X,t(X;9)(q28;q22). The mother showed heterozygosity for AR gene with two alleles (20 and 22 CAG-repeats) and non-random X-inactivation of the normal X in 93% of the cells.
Conclusion: As expected in carriers of balanced X-autosome translocations, the mother showed non-random XCI of the normal X. The der(X) in the daughter is expected to be non-randomly inactivated; the inactivation sometimes but not always spreads to the autosomal segment. The pathological phenotype of the proband appears to be due to partial or full duplication of the 9q22-qter segment.

P1050 - Cytogenetic and molecular profile of azoospermia in Algeria

Anissa Nini [1], Mostefa Bensaada [2], Maya Selsabil Khaled [3], El Chaima M'hemdi [3] , Rachid Rebiha [4]
Biotechnology Research Center, Genetic Diagnosis and Microscopy Laboratory, Health and Biotechnology Division, Constantine-Algeria [1] University of Khenchla, University of Khenchla, Khenchla-Algeria [2] Constantine 1 University, Constantine-Algeria [3], Medical Assisted Procreation clinical Center ROTABY, Algiers, Algeria [4]

Background: Non-obstructive azoospermia is diagnosed in approximately 10% of men with infertility in which no sperm is found in the ejaculate as a result of spermatogenesis failure.
Objective: The aim of this study was to evaluate the cytogenetic and molecular profile of patients with non-obstructive azoospermia in order to determine the prevalence of chromosome abnormalities and azoospermia factor (AZF) microdeletions.
Methods: 25 patients from the Algerian east with non-obstructive azoospermia (NOA) were included in this study out of 31 patients who were referred to the laboratory of genetic diagnosis at The National Biotechnology Research Center (CRBT). They all had a testicular biopsy and a hormonal assessment. They underwent cytogenetic analysis by standard G-banding; AZF microdeletions were examined by multiplex polymerase chain reaction (PCR) and capillary electrophoresis.
Results: Most of the patients had an abnormal level of hormones; a high level of FSH and LH with an average level of 18.29 mIU/mL and 8.89 mIU/mL respectively, a low level of testosterone with an average of 5.24nmol/L. Only SCO was correlated with the other parameters (Age: 0.35 FSH: 0.22 LH: 0.22 Testosterone: 0.094). All patients had a normal karyotype, and 8% of them were diagnosed with microdeletions
Conclusion: In our cohort we found that 8% of the infertile men with non-obstructive azoospermia had AZF microdeletions. Similar studies could be useful for genetic counselling of such patients.

P1054 - Cytogenetic and molecular characterization of a mosaic ring chromosome 13 mechanism of formation and instability

Mar Xunclà [1], Natàlia Rey [1], Neus Castells [1], Alberto Plaja [1], David Gómez-andrés [2], Pedro Antonio Martínez [1], María Serrano [1], Lourdes Trobo [1], Maria Àngels Rigola [3], Elena García-arumí [1], Eduardo Tizzano [1]
Hospital Vall D'hebron, Department of Clinical and Molecular Genetics, Barcelona-Spain [1] Hospital Vall D'hebron, Department of Pediatric Neurology, Barcelona-Spain [2] Universitat Autònoma De Barcelona, Department of Cellular Biology and Medical Genetics, Barcelona-Spain [3]

Background: Ring chromosomes are anomalies that imply loss of material and are characterized by their instability. During transmission through cell divisions, breakage-and-fusion processes occur, resulting in complex chromosomal anomalies.
Methods: G-band, chromosome painting and FISH cytogenetic analysis (Vysis), Array-CGH (8x60, ogt, UK).
Results: We present the case of a 10 year-old girl, daughter of consanguineous parents, with extreme short stature, microcephaly and minor dysmorphic features. A previous exome study had shown a terminal deletion at 13q33.3q34. Peripheral blood karyotyping revealed the presence in 80% of metaphases of a ring replacing a normal chromosome 13. The observed rings had two different sizes. FISH

study of chromosome 13 with LSI 13 probe (at 13q14 including the RB1 gene) showed signal in the large rings, but not in the small ones. Chromosome painting of chromosome 13 demonstrated that all the rings came from chromosome 13 with no other chromosome involved. A subsequent exonic CGH-array showed the presence in all cells of the terminal deletion already seen in the exome study, but also the presence of a 13q14.1q31.1 mosaic deletion that included the RB1 gene. The chromosome carrying these two deletions would be compatible with the small sized ring observed in the cytogenetic studies.
Conclusions: This case shows the evolution of a ring chromosome towards more complex anomalies due to breakage-and-fusion processes, as well as the need to combine different cytogenomic techniques to characterize complex chromosomal anomalies in detail, and to determine their origin and to suit a personalized clinical care.

P1055 - Currarino syndrome in two Moroccan siblings with inherited 7q36 deletion due to maternal t(7;21)(q36;p11) a case report

Zhour El Amrani [1], Abdelhafid Natiq [1], Aziza Sbiti [2], Ilham Ratbi [3], Thomas Liehr [4], Abdelaziz Sefiani [3], Maryem Sahli [2]
A. research Team In Genomics and Molecular Epidemiology of Genetic Diseases, Genomics Center of Human Pathologies, Faculty of Medicine and Pharmacy,, Medical Genetics, Rabat-Morocco [1] B. department of Medical Genetics, National Institute of Health In Rabat, Bp 769 Agdal, 10 090 Rabat, Morocco., Medical Genetics, Rabat-Morocco [2] A. research Team In Genomics and Molecular Epidemiology of Genetic Diseases, Genomics Center of Human Pathologies, Faculty of Medicine and Pharmacy, U, Medical Genetics, Rabat-Morocco [3] C. jena University Hospital, Friedrich Schiller University, Institute of Human Genetics, Am Klinikum 1, D-07747 Jena, Germany, Medical Genetics, Jena-Germany [4]

Background: Currarino syndrome (CS) is a rare disease characterized by the triad: presacral mass, anorectal malformation and sacral agenesis . Several incomplete forms of (CS) with variable phenotypes are described, since the only mandatory clinical feature for (CS) diagnosis is the sacral anomaly. Mutations in the gene MNX1, located in 7q36.3, were identified in nearly all patients with a familial form of Currarino syndrome and 30% of those with a sporadic form. On the other hand, large subtelomeric deletions in 7q36.3 region containing MNX1gene have also been identified in patients presenting the phenotype of (CS) and additional signs such as growth delay, facial dimorphism and intellectual disability.
Case presentation: We report the case of two siblings with incomplete form of Currarino syndrome combined with microcephaly and intellectual disability. Classical and molecular cytogenetic techiques revealed an unbalanced translocation der(7)t(7;21)(q36.2;p11.3)mat, with a deletion of the 7q36 region in both affected children.
Conclusion: This report highlights the importance of cytogenetics in diagnosis of rare genetic syndromes, with impact on genetic counselling of patients and their families.

P1056 - Prader Willi syndrome as a result of a de novo unbalanced translocation 15q;19p

Jasenka Wagner [1], Silvija Puseljic [2], Kristina Crkvenac Gornik [3]
Faculty of Medicine University of Osijek, Department of Medical Biology and Genetics, Osijek-Croatia [1] University Hospital Centre Osijek, Paediatric Clinic, Osijek-Croatia [2] University Hospital Centre Zagreb, Clinical Department for Laboratory Diagnostic, Zagreb-Croatia [3]

Prader-Willy syndrome (PWS – OMIM 176270) occurs in approximately 1 in 20,000 newborns, caused by the loss of paternally expressed genes in the imprinted 15q11-q13 chromosome region. Features of PWS are: central hypotonia, global developmental delay, short stature, genital hypoplasia, feeding difficulties during the neonatal period, changing to hyperphagia around the age of two. Mild dysmorphic features - mongoloid-set eyes, narrow forehead, triangular upper lip, small hands and feet. Mild to moderate mental retardation occurs in almost all individuals
We present a newborn male infant born in gestational week 35 by caesarean section due to abruption of maternal placenta. He was the third child of non-consanguineous parents aged over 40 years. Birth weight 2029 g, Apgar score 2/4, with respiratory distress syndrome. In the family a 3-year-old brother has an autism spectrum disorder. The phenotype of the patient included dolichocephaly, mongoloid eye position, high forehead, milder saddle nose root, gothic palate, microretrognathia, central hypotonia, genital hypoplasia and feeding difficulties, including a need for feeding through a nasogastric tube.
GTG banding showed de novo unbalanced translocation between 15q and 19p resulting in monosomy for 15q11-q13. His karyotype was: 45,XY,-15,der(19)t(15;19)(q12;p13.3)dn. aCGH analysis revealed an interstitial deletion of region 15q11.1q13.1 with a size of 8.96 Mb: arr[GRCh38] 15q11.1q13.1(19890228_28851998)x1. It is a Type 1 deletion (T1D) which extend from BP1 to BP3. The deleted region contains 26 genes, 10 of them are pathogenic: GABRA5, GBRB3, MKRN3, HERC2, OCA2, MAGEL2, NDN, NIPA1, SNRPN I UBE3A. MS-MLPA analysis showed that the deletion is on the paternal copy of chromosome 15. Analysis of chromosome 19 showed normal copy number pattern.
Up to date, a total of 15 cases of PWS as a result of a translocation have been described in the literature, and only two (Sun et al. 1996, Dang V et al. 2016)

involved chromosomes 15 and 19 with different sized deletions.

P1062 - Primary Ovarian Insufficiency don't neglect intragenic CNVs

Anna Lokchine [1], Anne Bergougnoux [2], Bénédicte Nouyou [1], Erika Launay [1], Laura Mary [1], Linda Akloul [3], Mathilde Domin-Bernhard [4], Sylvie Odent [3], Marc-antoine Belaud Rotureau [1], Elena Tucker [5], Sylvie Jaillard [1]
CHU Rennes, Laboratoire De Cytogénétique Et Biologie Cellulaire, Rennes-France [1] Université De Montpellier, Genetics and Pathophysiology of Neuromuscular Disorders, Phymedexp Research Unit, Cnrs, Inserm, Montpellier-France [2] CHU Rennes, Service de Génétique Clinique, Rennes-France [3] CHU Rennes, Service de Gynécologie, Rennes-France [4] University of Melbourne, Murdoch Childrens Research Institute, Melbourne-Australia [5]

Premature ovarian insufficiency (POI) is a major cause of female infertility. Currently, over 80 genes are reported to be responsible for POI. Our diagnosis strategy includes karyotyping, FMR1 screening, custom array-CGH focusing on POI genes, and whole exome sequencing (WES). Since 2019, over 200 patients benefited from this approach, with a diagnosis rate around 15% for pathogenic variants corresponding mainly to SNVs, identified in severe POI. Some SNVs of uncertain significance were upgraded to probably pathogenic after functional validation. for CNVs, our experience led us to conclude that intragenic variants may be underrated. We report the first case of intragenic compound heterozygous deletions in FSHR in a POI patient (primary amenorrhea, undeveloped breasts, atrophic genitalia). She inherited two intragenic FSHR deletions: one spanning at least from exon 5 to 9 (maternal) and one encompassing exons 3-6 (paternal). The breakpoints of the two deletions have been characterized by custom long-range PCRs followed by Sanger Sequencing. Eight loss-of-function deletions encompassing FSHR exons are currently reported in gnomAD SVs and none corresponds to the herein deletions. Only one homozygous FSHR deletion encompassing exons 2-10 has previously been reported in severe POI. In our case, a complete absence of DNA material corresponding to exons 4 and 5 of FSHR is sufficient to cause a severe phenotype. While the two novel FSHR in-frame deletions reported here are predicted to lead to shorter proteins with conservation of N-ter and C-ter domains, together they confer a very severe phenotype of FSH resistance, leading to consider every part of the FSHR as crucial for its function. Towards our cases and literature data, we show that intragenic CNVs in POI genes, using robust sequencing CNV pipeline or custom array-CGH, can lead to a conclusive molecular diagnosis and thus should not be neglected.

P1064 - Study of CNVs small rearrangements by CGH array on dedicated oligonucleotide chip in the setting of molecular diagnosis of Marfan syndrome related syndromes and non syndromic hereditary thoracic aortic aneurysms dissections (hTAAD).

Philippe Khau Van Kien [1], Aurélie Plancke [1], Guillaume Rolland [1], Caroline Beugnet [1], *Thierry Lavabre-Bertrand* [2]

CHU de Nîmes, UF de Génétique Médicale et Cytogénétique, Nîmes-France [1] CHU de Nîmes, Laboratoire de Cytologie Clinique et Cytogénétique, Nîmes-France [2]

Since 2011, our laboratory has routinely carried out molecular genetic analysis for the diagnosis of Marfan syndrome, related syndromes and non-syndromic hereditary thoracic aortic aneurysms/ dissections (hTAAD). We have developed a strategy based on NGS analysis of a panel of 50 genes involved in this group of diseases, with bioinformatics pipelines allowing the detection of point or small-size variants as well as CNVs. We also complemented this by CGH-array analysis on pangenomic oligonucleotide chips, highly enriched for the analysed genes. This is particularly useful to independently validate abnormalities detected by NGS analysis (corresponding sometimes to artefacts) or to confirm the absence of molecular abnormalities in a patient with strong clinical and/or familial arguments in favour of disease. In addition to the rearrangements identification and their delineation (intragenic, whole gene, contiguous genes syndrome), we here present our results involving the identification of recurrent CNVs among the 50 genes, such as the dup7q11.23 (ELN) and the dup16p13.11 (MYH11. Both CNVs have been recently associated with an increased risk of TAAD. Moreover and surprisingly, this strategy also allowed the differential diagnosis between Marfan syndrome and Klinefelter syndrome in 3 patients.
Overall, the combined strategy of using both cytogenomic and NGS analyses increases our diagnostic yield and reliability and allows us to give pertinent and comprehensive results to our corresponding physicians.

P1065 - Expanding the phenotype of 14q11.2 microdeletions encompassing CHD8 and SUPT16H genes

Anna Lengyel [1], Eva Pinti [1], Henriett Piko [2], Gyorgy Fekete [1], Iren Haltrich [1]
Semmelweis University, Pediatric Center Tuzolto Street Department, Budapest-Hungary [1] Semmelweis University, Department of Internal Medicine, Budapest-Hungary [2]

Microdeletions of chromosomal region 14q11.2 are associated with a phenotype including neurodevelop-

mental disorders [(NDDs); intellectual disability (ID), developmental delay (DD), autism spectrum disorder (ASD), other neuropsychologic disorders), macrocephaly, and characteristic facial features (hypertelorism, down-slanting palpebral fissures, broad nose, long philtrum, prominent Cupid s bow, abnormalities of the ears). A 35 kilobase large minimal critical region encompassing two genes – CHD8 and SUPT16H - has been identified. CHD8 is a well established ASD driver gene, is associated with a specific ASD sub-phenotype involving macrocephaly and gastrointestinal problems, and has recently been linked to schizophrenia and bipolar disorder as well. Variants in SUPT16H have been reported in patients with NDDs, seizures, precocious puberty, and corpus callosum abnormalities, but not macrocephaly.
In a Hungarian paediatric NDD cohort (N=78), three patients were identified as carriers of an overlapping ~500 kilobase large 14q11.2 microdeletion. All patients underwent detailed clinical evaluations; genetic testing included traditional G-banding and chromosomal microarray analysis. Comparison of our patients' phenotypes with those reported in the scientific literature and listed in online databases (n=12; at least 50% overlap and similar sized variants) enabled phenotypic expansion of the microdeletion: growth delay/short stature, muscular hypertonia, ventriculomegaly and hypertrichosis were novel features noted in 2/3 patients. Importantly, neither of our patients had macrocephaly; on the contrary, one child had microcephaly. Taken together with two further children reported to have normal head circumference, our results highlight the fact that macrocephaly is not an obligatory symptom in SUPT16H-CHD8 microdeletions.

P1071 - Interesting case studies in patients suffering from anorectal malformations

Drahuse Novotna [1], Marcela Malikova [1], Jana Drabova [1], Zuzana Slamova [1], Miroslav Stolfa [1], Hana Zunova [1], Anna Krepelova [1], Marketa Havlovicova [1]
2nd Medical Faculty, Charles University and University Hospital Motol, Institute of Biology and Medical Genetics, Prague-Czechia [1]

Anorectal malformations together with partial agenesis of sacrum and presence of pre-sacral mass are typical features of AD congenital Currarino syndrome, which is caused by pathogenic variants in the MNX1 gene located on chromosome 7q36.3. About 70 % of cases are familial but not all carriers of mutations are affected. Rarely, deletions of region 7q36.3 are described.
Using cytogenomics methods we have found different aberrations in patients suffering from anorectal malformations in addition to other inborn defects, abnormal phenotypic features and also intellectual disability (ID):
- a familial case of microdeletion of 7q36.3
- inv dup 4p16.3p15.2 del 4pter in a boy with hypotonia, club feet, sacral agenesis, spina bifida, hearing impairment, atrophy of both optic papillae and psychomotor delay
- inv dup 3p25.3p21.31 del 3p26.3p25.3 in a girl with Patau syndrome phenotype
- inv trp 3q26.3qter in a boy with Currarino syndrome, Blaschko´s lines, growth retardation and ID
- del 16q23.3q24.1 in a girl with VCC and respiratory failure (alveolar capillary dysplasia)
- male profile and mosaic duplication of 8q13.2q24.3 in a girl with VCC, abnormal genital and cleft palate
- del 7p22.3pter and dup 7q36.1qter in a girl with microcephaly and ID

In comparison to a group of 21 patients that have been diagnosed as positive by sequencing of MNX1 gene in 76 patients and their relatives, there were only two girls affected by any other inborn defects and two asymptomatic carriers of MNX1 gene mutation.
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P1072 - Synergy of various diagnostic methods in MCADD Slovenian patient

Maja Ficko [1], Daša Perko [1], Barbka ReppiČ Lampret [1], Ana Drole Torkar [2], Tinka Hovnik [1]
Umc, Clinical Institute for Special Laboratory Diagnostics, Ljubljana-Slovenia [1] Umc, Department of Pediatric Endocrinology, Diabetes and Metabolic Diseases, Ljubljana-Slovenia [2]

BACKGROUND
The medium chain acyl-CoA dehydrogenase (MCAD), encoded by the ACADM gene, is involved in mitochondrial fatty acid β-oxidation. MCAD Deficiency (MCADD) is the most common congenital disorder of fatty acid metabolism (OMIM # 201450) and is inherited in an autosomal recessive manner. MCADD is caused by homozygous or compound heterozygous changes in the ACADM gene at chromosomal region 1p31.1. Clinical symptoms present in MCADD are hypoketotic hypoglycaemia, vomiting, seizures and coma triggered by fasting, catabolic stress or common illness.
We present the case of a 2-year-old female patient with MCADD and the path to a final diagnosis.
METHODS AND RESULTS
Analysis of organic acids in urine using gas chromatography–mass spectrometry (GC/MS) showed the presence of hexanoylglycine and suberylglycine, laboratory markers of MCADD. Furthermore, the acylcarnitine profile, measured with tandem mass spectrometry (MS/MS), showed a characteristic pattern for MCADD, with elevated octanoylcarnitine (C8), decanoylcarnitine (C10), and decenoylcarnitine (C10:1).
However, next-generation sequencing revealed only one pathologic heterozygous change in exon 5 of the ACADM gene. It resulted in the substitution of the amino acid glycine to valin at position 118 (c.353G>T, p.Gly118Val). Further, the activity of MCAD in the lymphocytes of the patient was determined to be

below the limit of quantification (<0,02nmol/(min.mg protein)). This confirmed the diagnosis of MCADD, however, without a known genetic cause. The next step was to identify possible major changes on the second allele of the ACADM gene, which were not determined by sequencing. Additional MLPA analysis showed a heterozygous deletion of exon 1 and 2 of the ACADM gene of at least 3.9 kb in the chromosomal region 1p31.

CONCLUSIONS

We present a rare case of previously unpublished molecular genetic results combined with rare deletion of exon 1-2 in ACADM gene. The combined results are in accordance with the referral diagnosis and confirmed the deficiency of medium-chain acyl-CoA dehydrogenase.

P1078 - A case report of a child with 46 XX del(5)(q21.1q31.1) derived from a maternal insertion 46 XX ins(14;5)(q24.3;q21.1q31.1)

Tiiu Roovere [1], Kadi Jairus [1], Piret Ilisson [1], Gerly Kilusk [1], Laura Roht [1]
Tartu University Hospital, Genetics and Personalized Medicine Clinic, Tallinn-Estonia [1]

We report on a 2-month-old girl, who was referred to our clinic for genetic counseling due to dysmorphic phenotype and suspected hearing impairment. The clinical examination of the baby revealed dysmorphic features including clubfeet, unilateral dysmorphic ear, deep-set eyes, epiblepharon, broad nasal bridge, bulbous nasal tip, long fingers and bilateral single palmar crease. There was a slight hypertonia in the upper part of the body, otherwise her psychomotor development was normal at the time of the examination.

The girl was born from a twin pregnancy after an IVF treatment of non-consanguineous parents. The examination of family history revealed that the proband`s mother`s two sisters had died in early childhood: both had cleft palate and one of them had a horseshoe kidney.

Chromosomal microarray analysis of the proband revealed a large 32 Mb interstitial heterozygous deletion of the chromosomal region 5q21.1-q31.1. The deleted region contains over 80 protein coding genes, including the gene APC, which is associated with familial adenomatous polyposis-1 (FAP1). The patient was directed to regular FAP screening from her teenage years onwards.

Subsequent karyotype analyses of the patient´s family members revealed that her mother and twin brother are carriers of a cytogenetically balanced insertion 46,XX,ins(14;5)(q24.3;q21.1q31.1). The sisters of proband´s mother most probably were carriers of unbalanced forms, as 5q deletions are in concordance with their phenotypic findings.

Present case emphasizes the importance of gathering a thorough family anamnesis prior an IVF treatment and highlights the need for examining parental karyotypes for balanced aberrations in case of complicated family history.

P1079 - De novo intrachromosomal insertion with 1q32.1q32.2 duplication in a patient with neurodevelopmental delay

Isaltina Silva [1], Sílvia Pires [1], Nataliya Tkachenko [1], Natália Oliva-Teles [2]
Centro De Genética Médica Jacinto De Magalhães/centro Hospitalar Universitário De Santo António, Genetics, Porto-Portugal [1] Cgmjm/chupdsa/umib/itr/medicids, Genetics, Porto-Portugal [2]

Introduction: Chromosome insertions are rare events. Frequently, additional copy-number gains are observed in tandem; however further investigation is necessary to clarify the type of event and chromosomal regions involved. Phenotype consequences of insertion duplications depend on the over-expression of included dosage-sensitive genes and on the disruption or misregulation of genes spanning duplication and insertion breakpoints. The 1q32.1 duplication region includes many genes expressed in the brain, defining this region as critical for neurodevelopment.

Case description: A 19-year-old male patient with intellectual development disability, aggressive behaviour, attention deficit hyperactivity disorder, mild microcephaly and minor dysmorphic features including macrogenia was referred to the Cytogenetics Unit of our University Hospital Centre for conventional and molecular cytogenetics studies. His parents were non-consanguineous with unremarkable family history.

Results: Initial analysis of G-banded chromosomes showed a derivative chromosome 1 with additional material, apparently in 1q23.1. The karyotypes of the parents were normal. Microarray-CGH analysis of the patient showed a de novo copy number gain of chromosome region 1q32.1q32.2 of 8.1Mb, arr[GRCh37]1q32.1q32.2(200209305_208307371)×3.

Discussion: The rearrangement was interpreted as a de novo intrachromosomal insertion with 1q32.1q32.2 duplication, apparently in 1q23.1. Our findings are compared with previous reports with similar 1q32.1 duplications, which have been associated with intellectual disability, global development delay, dysmorphic features and other more variable phenotypic features (DOI: 10.1016/j.ejmg.2011.12.008; DOI: 10.1016/j.ejmg .2018.10.010). Although the microarray-CGH was essential to clarify the size and source of the duplicated region, the conventional karyotype was important to more accurately show the specific bands where the duplicated segment was inserted.

P1080 - Abnormal features of DiGeorge syndrome

Tereza Kutilova [1], Zuzana Slamova [1], Jana Drabova [1], Miroslav Stolfa [1], Tereza Kunikova [1], Lukas Ryba [1], Marketa Vlckova [1], Hana Zunova [1]
2nd Faculty of Medicine, Charles University and University Hospital Motol, Department of Biology and Medical Genetics, Prague-Czechia [1]

DiGeorge syndrome (DGS) is the most common microdeletion syndrome with a frequency of approximately 1:4000 live births and it is caused by heterozygous microdeletion of chromosome 22q11.2. The phenotype is highly variable and about 180 clinical manifestations have been described. Typical symptoms include congenital cardiac malformations, characteristic facial features, immune deficiency, and psychiatric disorders.
We present some carriers of 22q11.2 microdeletions with non-specific symptoms such as intellectual disability, epilepsy, developmental delay or ADHD. In these patients the aberrations were found by using array CGH. Case 1 is a twenty-six-year-old woman with mild intellectual disability, developmental delay and epilepsy, who does not manifest any other typical symptoms for DGS. Case 2 is an eight-year-old boy with ADHD, developmental delay, delay of speech and cognitive development. In addition, he has a heart defect, which was detected after diagnosing DGS. Case 3 is a seven year-old boy with developmental delay, speech delay, mild intellectual disability and growth delay. Case 4 is a nine-year-old girl with inflammatory bowel disease, growth delay and no heart defect. She has deletion of 22q11.2, which does not affect any critical genes for DGS. Her brother with learning difficulty carries the same deletion. They inherited the aberration from their father suffering from immunodeficiency.
From our outcomes it is obvious that the expressivity of DGS is more variable than supposed. A second-hit model could explain such variability

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P1085 - A case of mosaicism with a complex unbalanced reciprocal translocation and a normal cell line in a male patient with fertility problem. A challenging cytogenomic diagnosis.

Eva-Maria Krimmel [1], Uwe Heinrich [1], Wilena Telman [1], Dagmar Wahl [2], Konstanze Hörtnagel [3]

MVZ Martinsried, Cytogenomics, Martinsried-Germany[1] Medicover Humangenetik - Augsburg, Clinical Genetics, Augsburg-Germany[2] MVZ Martinsried, Clinical Genetics, Martinsried-Germany[3]

A married couple (41-year-old woman and 50-year-old man) with a history of a previous miscarriage, an ectopic pregnancy and a healthy 5-year-old son was referred for chromosomal analysis due to the desire to have more children. The wife showed an unremarkable female set of chromosomes (karyotype: 46,XX). The chromosome analysis of the husband revealed an apparently normal male karyotype in about 73% of the 30 metaphases examined and a karyotype with a structurally changed derivative chromosome 6 and a structurally changed derivative chromosome 13 in the remaining 27% of the cells. To exclude a possible culture based event the chromosome analysis was repeated with a new blood sample and the previous result could be confirmed.
Cytogenetically, an unbalanced reciprocal (6;13) translocation between the long arms of the derivative chromosome 6 and the derivative chromosome 13 was suspected (karyotype: 46,XY,der(6)t(6;13)(q?; q?), der(13)t(6:13)(q?;q?)). The reciprocal translocation could be confirmed by means of FISH analysis. Subsequent array-CGH analysis revealed a loss of the chromosomal region 13q14.2-q31.1 and an approximately 260 kb loss in band 6q27, both in mosaic form. Since the cytogenetic and molecular-cytogenetic methods carried out so far did not provide precise information on neither the localization of the breakpoints nor the position and size of the deleted region in the translocation segment of chromosome 13, optical genome mapping (OGM) was carried out on ultra-high molecular weight DNA from the patient's EDTA blood; the result of which is still pending and will be presented in the poster. Due to the mosaic constellation of the structural abnormalities a statement on the clinical relevance is not possible.

P1088 - Intrachromosomal insertion or paracentric inversion A classic trap in chromosome analysis

Samira Ahmed-Elie [1], Anaïk Previdi [1], Aziza Lebbar [1], Martine Montagnon[1], Justine Zini[2], Audrey Benezit[3], Jean-Michel Dupont[1]
Hôpital Cochin - Aphp Paris Centre - Université Paris Cité, Service De Médecine Génomique Des Maladies de Système et d'organe, Paris-France [1] Hôpital Raymond Poincaré - Aphp Paris Île-de-france Ouest – Université De Versailles Saint-Quentin-en-Yvelines, Service De Service De Soins De Réadaptation Post-réanimation - Pôle Pédiatrie, Paris-France [2] Hôpital Raymond Poincaré - Aphp Paris Île-de-france Ouest - Université De Versailles Saint-Quentin-en-Yvelines, Service De Réanimation Infantile – Rééducation Neuro-respiratoire - Pôle Pédiatrie, Garches-France [3]

Intrachromosomal insertions are very rare chromosomal events which may be misinterpreted as paracentric inversions.
We report here a 1-year old child born from healthy non-consanguineous parents, who was referred to the hospital for developmental delay associated with axial hypotonia.
Chromosomal Microarray Analysis revealed a gain of 6,58 Mb of the 16p13.3 region. An abnormal short arm of chromosome 16 was observed at karyotype and

fluorescent in-situ hybridization confirmed a gain of the distal part of chromosome 16 short arm. Karyotype of parents showed that the duplication was an unbalanced recombinant of a maternal rearrangement, interpreted at first glance as a paracentric inversion. However, the absence of an associated deletion in the proband, which would be expected with any recombinant chromosome derived from an inversion, led us to consider the alternative hypothesis of a maternal intrachromosomal insertion.
Using several BACs localized along chromosome 16 short arm, we could validate this hypothesis by classical chromosome walking approach. We took advantage of the new genome optical mapping technology by BIONANO® to further confirm this abnormality in a straight forward approach with a higher resolution.
This case illustrates the importance of combining different cytogenetics methods to clearly define the rearrangement which is mandatory for the medical follow-up of the families
The identification of this insertion was paramount to refine the recurrence risk, allowing for an accurate parental genetic counseling.

P1089 - Optical mapping characterization of a very complex chromosomal insertion

Alberto Plaja [1], Neus Castells [1], Mar Xunclà [1], Natalia Rey [1], Anna María Cueto-González [1], Maria Obón [2], Irene Mademont [2], Maria Angels Rigola [3], Jessica Tijero [4], Elena Garcia-Arumí [5], Eduardo Tizzano [5]
Hospital Universitari Vall D'hebron, Department of Clinical and Molecular Genetics, Barcelona-Spain [1] Hospital Universitari De Girona Doctor Josep Trueta, Department of Clinical Genetics, Girona-Spain [2] Universitat Autònoma De Barcelona (uab), Department of Cellular Biology and Medical Genetics, Bellaterra., Barcelona-Spain [3] Josep Carreras Leukaemia Research Institute, Microarrays Unit, Barcelona-Spain [4] Hospital Universitari Vall D'hebron, Department of Clinical Genetics, Barcelona-Spain [5]

Introduction: Complex chromosomal rearrangements are difficult to characterize with classical molecular cytogenetic techniques. We present a woman with a very complex insertional rearrangement and siblings showing a surprisingly variety of chromosome imbalances revealed by array CGH
-A girl with intellectual disability, developmental delay and short stature with two regions deleted at 9q21.13q21.32 and 9q21.33q22.31 separated by a normal dose region.
-A boy with mild intellectual disability and two duplications at 9q21.13q21.32 and 9q21.33q22.31 separated by a normal dose region
-A fetus with a continuous 9q21.13q22.31 duplication not interrupted by normal regions.
Methods and results: Conventional G-band karyotype, FISH and array CGH techniques showed an interstitial insertion of a chromosome 9q segment in 3q. This information does not provide a full explanation of the chromosomal imbalances observed in the siblings.
Optical mapping revealed in the mother a very complex event consisting of insertion of 9q21.13q21.32 and 9q21.33q22.31 fragments into chromosome 3q22.1, and two additional insertions of 9q21.32 and 9q21.33 regions into 9q22.1, providing a full explanation of all the chromosomal imbalances observed in this family.
Conclusions: This case demonstrates the high diagnostic capacity of optical mapping techniques: a single test allowed a complete characterization of a complex organization that could not be achieved by applying several molecular cytogenetic conventional assays.

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P1092 - Rare genomic imbalances encompassing kinase genes in a group of patients with autism spectrum disorders

Magdalena Budisteanu [1], Sorina Mihaela Papuc [2], Alina Erbescu [2], Lucian Albulescu [3], Florina Rad [4], Gisela Gaina [5], Laura Mateescu [4], Diana Barca [6], Maria Dobre [7], Emanuela Lucia andrei [1], Bogdan Budisteanu [4], Adelina Glangher [1], Florentina Linca [1], Doina Ioana [1], Sorin Riga [8], Aurora Arghir [9]
Prof. Dr. Alex. Obregia Clinical Hospital of Psychiatry, Psychiatry Reasearch Laboratory, Bucuresti-Romania [1] Victor Babes National Institute of Pathology, Medical Genetics Laboratory, Bucharest-Romania [2] Victor Babes National Institute of Pathology, Biochemistry, Bucharest-Romania [3] Prof. Dr. Alex. Obregia Clinical Hospital of Psychiatry, Child and Adolescent Psychiatry, Bucharest-Romania [4] Victor Babes National Institute of Pathology, Cell Biology and Experimental Myology, Bucharest-Romania [5] Prof. Dr. Alex. Obregia Clinical Hospital of Psychiatry, Pediatric Neurology, Bucharest-Romania [6] Victor Babes National Institute of Pathology, Molecular Pathology, Bucharest-Romania [7] Prof. Dr. Alex. Obregia Clinical Hospital of Psychiatry, Stress Research Laboratory, Bucharest-Romania [8] Victor Babes National Institute of Patholog, Medical Genetics Laboratory, Bucharest-Romania [9]

Background: Autism spectrum disorders (ASDs) are complex and heterogeneous neurodevelopmental conditions. Rare copy number variants (CNVs) and sequence changes have a well-recognized contribution to ASD pathogenesis. Among the involved genes, kinases encode for proteins with important biological roles including brain development and function.
We report the results of array-based genomic comparative hybridization (array-CGH) and triplet primed PCR/MS-MLPA screening for fragile X syndrome, in a group of ASDs patients, with focus on defects involving kinases.
Methods: 300 patients with ASDs were included in this study, after a complex clinical workup including

neurological, psychiatric, and psychological evaluations. The FMR1 promotor was screened for abnormal methylation (MS-MLPA) and trinucleotide repeat expansion (TP-PCR), for male patients only. Array-CGH was performed in all ASD patients and a similar number of children with no neuropsychiatric diagnosis, using SurePrint G3 Human CGH microarray (Agilent Technologies).
Results: Rare exonic CNVs (< 1% of the total sample) were detected in 200 out of 300 patients and full mutation of the FMR1 in four. Thirty-two kinases were encompassed by rare exonic CNVs. Among these kinases, nine are considered causal for specific conditions and five belong to a larger genomic region associated with known syndromes, such as 1q21.1 duplication, 3q29 deletion, Jacobsen syndrome, 22q 11.2 duplication and Xq28 duplication - Lubs type syndrome.
Conclusions: Taking into account the genetic link of kinases with neurodevelopmental disorders, the increasing evidence that kinase dysfunction may play a causative role in these conditions, and the fact that kinases represent promising therapeutic targets, new patient data are needed to enhance the existing knowledge on kinase contribution to ASD pathophysiology.
Grants: The research leading to these results has eceived funding from the EEA Grant 2014-2021, under the project contract No 6/2019
References: Nourbakhsh et al 2021 (doi: 10.3389/fncel.2021.624648)

P1095 - Optical Genome Mapping (OGM) Validation and characterization of marker chromosomes

Cristina Pérez [1], Eli Lloveras [1], Begoña Méndez [1], Susana Martín [1]
Barcelona Central Lab. Synlab International Group., Genetics-cytogenetics and Cytogenomics, Esplugues De Llobregat-Spain [1]

Introduction: Optical genome mapping (OGM) is an emerging technology that has demonstrated its ability to detect all classes of structural variants (SVs), including copy number variations (CNVs) and unbalanced and balanced anomalies among others, in a single essay.
OGM is presented as a new diagnostic tool to examine patients with genetic abnormalities as markers chromosomes not detected or fully characterized with current routine methodologies.
Patients and Results:
Patient 1: Female 3-year-old patient followed up for developmental language disorder.
A chromosomal study was performed with the presence of a marker in all cells. An array was performed to better characterization showing a tetrasomy gain: arr[GRCh37] 13q11q12.12(1943628 _248894 82)x4.
A OGM study was carried out (Bionano Genomics Saphyr® and Bionano Access software, following the manufacturer's protocols) consistent with the result of the array. Also, the OGM allowed characterizing the gain as a chromosome 13 marker and its gene content.
Patient 2: Female 10-year-old patient referred because of intellectual disability, associated ASD and ADHD.
A chromosomal study showed the presence of a complex mosaic karyotype involving four different markers. FISH, MLPA and array techniques were performed in order to identify the chromosomes markers resulting in gains of chromosomes 4, 5, 8 and 13.
OGM was performed confirming all copy number gains detected on chromosomes 4, 5, 8, and 13 and allowing characterization of the chromosome markers detected with the previous techniques.
Conclusions:
- OGM emerges as a technology able to completely reveal the identity, gene content and complexity of marker chromosomes.
- OGM allows having all this information in a single and simple workflow, improving the time to results and the economic impact that it implies.

P1096 - Detection of 12;13 reciprocal translocation with copy number losses detected by karyotype and array and characterized by Optical Genome Mapping.

Elisabet Lloveras [1], Begoña Mendez [1], Susana Martin [1], Cristina Perez [1]
Barcelona Central Lab. Synlab International Group., Genetics Departament-cytogenetics and Cytogenomics, Esplugues De Llobregat-Spain [1]

Introduction: Balanced chromosome abnormalities, including translocations and inversions, are structural rearrangements of genetic material with no overall gain or loss detected with conventional karyotyping. However, about 6% of de novo translocations are associated with an abnormal phenotype. Sometimes, because the rearrangement disrupts a dosage-sensitive gene, separates a gene from its cis regulatory elements, or generates a functional chimeric gene. and sometimes because cryptic genomic imbalances, related or not to the breakpoints, have been found using genome-wide microarrays. Recently, a new technology, Optical genome mapping, has demonstrated its ability to detect all classes of structural variants including copy number gains and losses, in a single assay.
We present one case with apparently balanced translocation detected by karyotype, with copy number losses detected by array and characterized by optical genome mapping.
Patient and methods: The present case is a five-year-old girl evaluated in the Pediatric Neurology consultation. Slight dysmorphia, motor and language difficulties, and ASD suspicion was detected. Karyotype, array and optical genome mapping were performed. Karyotype detected a balanced translocation 12;13 and array a copy number loss in chromosomes 12 and 13. Optical genome mapping

detected the rearrangement and copy number losses and highlighted the genes disrupted by the rearrangement and the localization of the copy number losses.
Comments: Optical genome mapping is a new diagnostic tool for characterizing apparently balanced reorganizations detected in affected carriers.

P1100 - ICF Syndrome diagnosis conventional cytogenetics could be the key to disclose the syndrome

Eunice Matoso [1], Sónia Lemos [2], Joana Serra Caetano [3], Alexandra Estevinho [4], Rita Cerqueira [5], Sérgio B Sousa [6], Jorge M Saraiva [7]
Faculty of Medicine, University of Coimbra, Centro Hospitalar e Universitário de Coimbra, Cytogenetics Laboratory, Medical Genetics Unit; Icbr- Cimago/ cacc, Coimbra-Portugal [1] Centro Hospitalar E Universitário De Coimbra, Hospital Pediátrico, Primary Immunodeficiency Consult, Serviço De Pediatria Ambulatória, Coimbra-Portugal [2] Centro Hospitalar E Universitário De Coimbra, Department of Paediatric Endocrinology, Diabetes and Growth, Coimbra-Portugal[3] Centro Hospitalar e Universitário de Coimbra, Hospital Pediátrico, Cytogenetics Laboratory, Medical Genetics Unit, Coimbra-Portugal [4] Cgc Genetics, Molecular Diagnostics and Clinical Genomics, Porto-Portugal [5] Centro Hospitalar E Universitário De Coimbra, Hospital Pediátrico, Medical Genetics Unit, University of Coimbra, Faculty of Medicine, University Clinic of Genetics, Coimbra-Portugal [6] Centro Hospitalar e Universitário de Coimbra, Hospital Pediátrico, Medical Genetics Unit, University of Coimbra, Faculty of Medicine, University Clinic of Pediatrics, Clinical Academic Centre of Coimbra, Coimbra-Portugal [7]

Immunodeficiency, with centromeric instability and facial anomalies (ICF syndrome) is a very rare autosomal recessive disorder. It is characterized by reduced immunoglobulin levels, instability of pericentromeric regions of chromosomes 1 and 16 (and sometimes 9) upon mitogen stimulation of T-lymphocytes and mild facial dysmorphism. Pericentromeric instability is the consequence of DNA hypomethylation, not only present in pericentromeric repeats, but also in subtelomeres (ICF1, MIM# 242860), or in centromeric α-satellite repeats. Herein we report a patient with ICF syndrome revealed by conventional cytogenetics analysis.
We report a 15-year old boy, referred for conventional cytogenetics because of short stature and hypogonadotropic hypogonadism. Synchronized cell cultures of peripheral blood mitogen-stimulated T-lymphocytes were setup, GTL and CBG banding techniques were performed and 50 metaphases scored or analyzed between 550-750 bands level resolution.
Although the constitutional karyotype was 46,XY, 16% of the metaphases showed multiradiate/ branching of the long arms of chromosomes 1 and 16, as well as breaks and pericentromeric decondensation. These findings and the clinical evidence of long term hypoimmunoglobulinemia with recurrent respiratory infections, led to the most probable diagnosis of ICF Syndrome. Parents are second cousins. An earlier study on the patient with a NGS immunodeficiency panel, based on WES, gave no relevant results. Following the cytogenetics report WES reanalysis was conducted for the genes already known to be associated with the ICF syndrome: DNMT3B (ICF1), ZBTB24 (ICF2), CDCA7 (ICF3), and HELLS (ICF4). A homozygous missense variant was found in the HELLS (helicase, lymphoid specific) gene (NM_018063.5 c.2062A>G p.Thr688 Ala), probably a VUS (variant of uncertain significance). These promising results led to DNA methylation episignature investigations. In conclusion, conventional cytogenetic analysis could be the key to identifying the ICF syndrome and can lead to a more accurate diagnosis

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P1103 - Recurring phenotype in a family A coincidence or not

Eva Pinti [1], Anna Lengyel [1], Lena Szabo [1], Gyorgy Fekete [1]
Semmelweis University, Pediatrics, Budapest-Hungary [1]

In the Neurology Department we examined a three-and-a-half-year-old boy with the following symptoms: epilepsy, dysarthria, psychomotor developmental delay, generalized hypotonia, joint hypermobility, hyporeflexia, partial agenesis of corpus callosum, hippocampal malrotation, brachycephaly, narrow forehead, broad thumbs. He had one healthy brother and another brother with similar symptoms (moderate intellectual disability, brachycephaly, narrow forehead, generalized hypotonia, joint hypermobility, hyporeflexia), and a maternal, ten-year-old female cousin with moderate intellectual disability. His symptomatic brother previously underwent whole exome sequencing in another hospital, which detected a variant of uncertain significance in the X-chromosomal USP9X gene. His unique, hitherto undescribed variant was classified as likely pathogenic because of its poorly tolerated effect on protein function according to protein prediction software and the overlapping phenotype of the boy and the USP9X related disease. According to targeted polymorphism testing the index patient with epilepsy had the same variant, but the female cousin, the mother and the healthy brother did not carry the gene alteration. This family's case was an illustrative example for germline mosaicism of the USP9X likely pathogenic variant in the mother. Further conclusion was the independent occurrence of the maternal cousin's non-syndromic intellectual disability, because it is likely caused by various non-genetic effects.

P1109 - Complete paternal isodisomy of chromosome 15 in a patient with atypical presentation of Angelman syndrome

Oğuz Çilingir [1], Elif Saraç [1], Sabri Aynaci [1], Sinem Kocagil [1]
Eskişehir Osmangazi University, Medical Genetics, Eskişehir-Turkiye [1]

Angelman syndrome (AS) is a severe neurodevelopmental disorder characterized by intellectual disability, ataxia, absence of speech, seizures and microcephaly. Affected children present happy, sociable behavior and subtle dysmorphic features such as deep-set eyes, wide mouths and pointed chins. Paternal UPD (pUPD) of 15q11-12 region is one of the rarest (2-7%) underlying causes, with less than 50 patients reported in the literature and clinical features overlapping with Prader-Willi syndrome have been observed among pUPD AS.
Here we report a 19 month-old male patient with microcephaly, neuromotor developmental delay, and dysmorphic features. He was a hypotonic infant; gaining his head control at 6 months and sitting without support at 10 months. He had a speech delay and could walk with assistance. He did not have any seizures and his EEG was normal. In his physical examination, his weight was 12,6 kg (0,5 SD), height was 83 cm (-0,34SD), and head circumference was 44,8 cm (-2,4 SD). His dysmorphic features included narrow forehead, almond shaped eyes, thin upper lip and wide mouth. Prader- Willi syndrome was suspected in the patient initially, but karyotype analysis and 15q11-13 FISH analysis was normal. Microarray analysis showed a loss of heterozygosity of 77,892,262 bp at the 15q11.2q26.3 region. MS-MLPA assay showed hypomethylation of MAGEL2-1, SNRPN3, UBE3A1, SLC9A2-2, ITSN1-12 regions, suggesting the absence of maternal imprinting and, therefore, AS.
pUPD AS patients are expected to be mildly affected compared to deletion/mutation types, but still, most of the patients are reported to have abnormal EEG, seizures, and stereotypical movements such as hand clapping in contrast to our patient. It should be kept in mind that due to highly complex nature of the imprinting disorders, rare mechanisms can lead to overlapping clinical features.

P1111 - A de novo small marker chromosome that causes Trisomy 9p in a patient with failure to develop, microcephaly and normal neuromotor development

Sevilhan Artan [1], Derya Hazal Ozbakir [1], Efsun Tosumoglu [1], Koray Harmanci [2], Gülcin Panal [1], Ebru Erzurumluoglu Gökalp [1]
Eskisehir Osmangazi University, Medical Genetics, Eskisehir-Turkiye [1] Eskisehir Osmangazi University, Pediatric Immunology and Allergy, Eskisehir-Turkiye [2]

Trisomy 9p or Rethore syndrome is the fourth common structural chromosome anomaly detected in infantile period. Since it is an anomaly involving a region with a low gene content, it is thought to be compatible with life, therefore, more frequently seen in the population. It is characterized by partial or complete duplication of the short arm of chromosome 9, and clinical findings include failure to thrive, microcephaly, neuromotor developmental delay, and dysmorphic features.

Here we report 14 months old male patient who was referred to our outpatient clinic for failure to thrive, microcephaly, and facial dysmorphism. He was born 2740 gr at 37th week of gestation from nonconsanguineous parents. He had rotavirus infection when he was 9 months old and has had recurrent respiratory infections since then. At his echocardiography secundum atrial septal defect was detected. His neuromotor developmental milestones have been normal so far. At his physical examination, his weight was 7850 gr (-1,9SD), height was 72,5 cm (-1,17SD), and head circumference was 43,5 cm (-2,31SD). He had a narrow forehead, thin eyebrows, deep-set eyes, upslanted palpebral fissures, retrognathia, simian crease at the right hand and 2-3 toe syndactyly. Cytogenetic and locus-specific FISH analyses of the patient revealed 47,XY,+der(9)(9pter->9q12).ish der(9)(CDKN2Ax3, CEN9x3, 9q21x2)dn. Microarray analysis revealed a complete heterozygous duplication of the short arm of the chromosome 9 and reported as arr[GRCh37] 9p24.3p12 (46587_41983937)x3.
Trisomy 9p is a relatively common chromosomal anomaly, but most patients reported so far have a parent with a balanced chromosomal rearrangement. To our knowledge this is the first case of isolated trisomy 9p occuring as a small marker chromosome.

P1121 - Molecular confirmation of PHEX related hypophosphatemic rickets in a nine year old girl

Anette Eek [1], Anne Signe Bø [1], Lene Johannessen [1], Kristian Tveten [1], Chaerlotte Von Der Lippe [1], Trine Prescott [1], Bojovic Ognjen [2], Cathrine Heier [3], Elisabeth T. Ulseth [4]
Telemark Hospital, Medical Genetics, Skien-Norway [1] Haukeland University Hospital, Medical Genetics, Bergen-Norway [2] Oslo University Hospital, Children's Department, Oslo-Norway[3] Sørlandet Hospital, Children's Department, Kristiansand-Norway [4]

Genetic testing was requested for a nine year old girl diagnosed with hypophoshatemic rickets based on pathognomic biochemical and radiological findings. Molecular confirmation of the diagnosis was requested in conjunction with application for gene-specific therapy with Crysvita (burosumab).
High-through-put sequencing (HTS) for a gene panel for hypophosphatemic rickets was performed without resultant pathogenic or likely pathogenic findings.

The same gene panel was investigated using a 1M array CGH (Agilent) which detected a heterozygous deletion of two probes in intron 1 of PHEX. The analysis could not determine whether exon 2 was included in the deletion. PHEX specific MLPA (P223, MRC Holland) revealed a heterozygous deletion of the probe for exon 2, confirming a deletion in PHEX encompassing intron 1 and exon 2. Exonic deletions in PHEX gene are a known cause of X-linked dominant hypophoshatemic rickets, and this result confirms the child's clinical diagnosis.

P1122 - Ring chromosome 13 and translocation of 13q31.1 qter to 21p12 in a healthy female with medical history of eye cancer

Sylke Singer [1], Ruth J. Falb [1], Joohyun Park [1], Cristiana Roggia [1], andreas Dufke [1], Natalia Prodan [2], Günther Rettenberger [3], Ulrike A. Mau-Holzmann [1]
Institute of Medical Genetics and Applied Genomics, University of Tuebingen, Tuebingen-Germany[1] Department of Obstetrics and Gynecology, University of Tuebingen, Tuebingen-Germany [2] Genetikum, Genetic Counseling and Diagnostic, Neu-Ulm-Germany [3]

We report a healthy female carrying a ring chromosome 13 and a derivative chromosome 21 with translocated 13q-material on the short arm.
A 23-years-old primipara was referred for prenatal examination at 11 weeks of gestation due to multiple ultrasound abnormalities (hydrops fetalis, increased nuchal translucency, ventriculomegaly and signs of spina bifida). Cytogenetic analysis of chorionic villi showed a ring chromosome 13. Prenatal array analysis revealed a deletion of 32,3 Mb from 13q31.1 to 13q34 in 80% and a monosomy 13 in 20 % of cells. The pregnancy was terminated.
Surprisingly, chromosome analysis of our patient revealed a balanced karyotype: besides the ring chromosome 13, she is carrier of a structurally abnormal chromosome 21 - the material missing from ring chromosome 13 was found on the short arm of a chromosome 21. The findings were confirmed by FISH analysis, and she was karyotyped as 46,XX,r(13)(::p11-->q31.1::),der(21)(13qter-->13q31.1::21p12-->21qter). The rearrangement seemed to be balanced.
Interestingly, both our female patient and her mother suffered from unilateral eye tumor in early childhood. Unfortunately, no medical records were available for both tumors. FISH analysis with the RB1-probe in 13q14 showed no deletion of the RB1-locus in our patient. Due to familial history strongly suggestive for retinoblastoma, further analyses (sequencing and methylation analysis) of RB1 have been initiated. Additionally, karyotyping of the mother is planned. The father is unfortunately not available.
These analyses will hopefully reveal further insights into the eye tumors in the family as well as into the possible inheritance of the structural aberration.

P1126 - Assessing variants of uncertain significance a retrospective analysis of aCGH cases

Inês Costa [1], Joel Pinto [1], Ana Paula Neto [1], Carolina Almeida [1], Sofia Dória [1]
Faculty of Medicine, University of Porto, Department of Pathology-Genetics, Mangualde-Portugal [1]
Background: Array comparative genomic hybridization (aCGH) is considered the gold standard test for neurodevelopment disorders and congenital abnormalities. We aimed to reanalyse our Variants of Uncertain Significance (VUS) classified over a 10-years period.
Methods: VUS were selected from the aCGH database of the Genetics Department, Faculty of Medicine, University of Porto. The database includes 3166 patients studied from 2012 to 2022. The aCGH was performed using the Agilent 4x180K platform. The main clinical information from all the patients was also collected.
Results: 3244 VUS were identified in 1938/3166 patients (61,2%). In 1623 cases only VUS were identified. In 315 cases, VUS were found additionally to a pathogenic/likely pathogenic variant. The main clinical indications were autism spectrum (25,61%), followed by psychomotor development disorders (18,56%). Chromosome with the highest frequency of VUS was chromosome X (12,58%) with 203 and 205 VUS respectively overlapping the short and the long arm respectively, followed by chromosome 7 with a frequency of 7,43% overlapping mainly the long arm. Chromosome 21 registered the lowest number of VUS with a frequency of 1,82%.
From the 3244 VUS, 440 were selected to be reanalysed considering they were present at least in two different patients. From these, 71 VUS were reclassified as benign/likely benign variants. Correlations between frequent VUS and similar clinical indications were addressed.
Discussion:
Despite the continuous databases updating, there is a significant inter-laboratory discrepancy in the classification of genetic variants. Within a laboratory, classification also changes over the years. Regular update of variant databases is crucial to better define the clinical significance of VUS. Reporting these classification changes will allow improving the genetic counselling with a higher impact particularly in the context of prenatal diagnosis, reducing the anxiety associated with VUS reporting.

P1132 - Cytogenetic and flow cytometric findings in skin fibroblasts of patient with FANCI subtype of Fanconi anemia

Anna Repczynska [1], Katarzyna Julga [1], Ewelina Lazarczyk [1], Julia Radon-proskura [2], Reinhard Kalb [3], Olga Haus [1]
Department of Clinical Genetics, Faculty of Medicine, Collegium Medicum In Bydgoszcz, Nicolaus Coperni-

cus University In Torun, Department of Clinical Genetics, Bydgoszcz-Poland [1] Department of Pediatrics, Hematology & Oncology, Faculty of Medicine, Medical University of Gdańsk, Department of Pediatrics, Hematology & Oncology, Gdansk-Poland [2] Arbeitsgruppe Somazellgenetik, Institut für Humangenetik, Universität Würzburg, Institut für Humangenetik, Würzburg-Germany [3]

Background: Highly variable clinical symptoms of Fanconi anemia (FA), and the overlap of other syndromes make the diagnosis problematic. Analysis of chromosome breakage test with mitomycin C (MMC) and cell cycle by using flow cytometry best reveal characteristic changes in FA cells and allow confirmation of the clinical diagnosis. If the result of the MMC test from blood lymphocytes is equivocal or hematopoietic somatic mosaicism is suspected in the patient, the analysis of skin fibroblasts is required.
Patient: A 14 years old girl presented with short stature, dysmorphic features and café au lait spots was admitted to the genetic counselling clinic. Clinical diagnosis of FA was established.
Methods: Cytogenetic studies were carried out on peripheral blood lymphocytes and on skin fibroblasts from cultures supplemented with MMC. Chromosome instability data were analyzed and calculated. for cell cycle analyses nuclei were stained with DAPI. The DNA content was determined using a LSRII flow cytometer.
Results: The result of MMC test on lymphocytes was not fully supported clinical diagnosis. There was only slightly increased level of chromatid/chromosome breaks and a few metaphase spreads with radial figures and 'railroads chromosomes'. In contrast, the results of MMC test on skin fibroblasts were unequivocal. They revealed that ~90% of cells were damaged. Functional testing of fibroblasts by using flow cytometry indicated hypersensitivity to MMC in the form of an increase of the G2 phase fraction, which confirmed cytogenetic results. Eventually, mutation testing was performed, revealing a biallelic mutation in FANCI gene.
Comment: This case shows that when MMC test on lymphocytes is equivocal like in our patient, the fibroblast MMC test and cell cycle analysis can be the methods which allow the final adequate diagnosis to be established.

P1137 - Investigation of Genetic Etiology of Short Stature

Birsen Karaman [1], Adam Najafli [1], Firdevs Bas [2], Asli Kalkandelen [2], Feyza Darendeliler [2]
Istanbul University, Medical Genetics, Istanbul-Turkiye [1] Istanbul University, Endocrine and Metabolism, Istanbul-Turkiye [2]

Slowing down or pausing growth often indicates a health problem in the child. The main criteria of short stature are the third height percentile, growth rate below the 25th percentile, height 8.5 cm shorter than the expected one, and bone age older than that of chronological age (>2 SD). Medical history, physical examination, and laboratory tests are of great importance in the investigation of short stature, and these procedures significantly contribute to elucidating etiologic causes.
Short stature is divided into two categories, clinically pathological and non-pathological, divided into subgroups related to genetic causes: chromosomal, single gene, and multifactorial factors.
This study evaluated the genetic etiology of 189 clinically diagnosed short-stature patients with unknown underlying causes using cytogenetic and molecular tests to elucidate the genetic etiology. Patients with normal karyotypes were included in the analysis of 10 genes (GH1, GHR, GHRH, GHSR, IGF1, IGF1R, IGFALS, IGFBP3, SHOX, and STAT5B) which was carried out on the Ion Torrent platform. Mutations thought to have clinical importance were confirmed by Sanger sequencing, and family studies were used for further investigation.
In five cases, significant mutations (BMP4, GHR, IGSF1, LHX4, and PROKR2) were identified, only one of which was previously reported. Three mutations were also detected in healthy family members.
This panel of short stature-associated genes was designed for the first time in Turkey. Its results are expected to elucidate genetic factors that play a role in the etiology of this phenotype, reveal genotype-phenotype correlations, and contribute to personalized genetic counseling. But, since short stature has a broad genetic background, we think a gene panel is insufficient. Using whole-exome or whole-genome sequencing, the proper approach would be to investigate index individuals, their parents, and healthy controls.

P1142 - Challenges detecting a MAGEL2 in frame deletion variant two clinically distinct families and sequencing issues

Sarah Delbaere [1], Jo Sourbron [1], Erika D'haenens [1], Toon Rosseel [1], Eveline Debals [1], Machteld Baetens [1], Annelies Dheedene [1], Olivier Vanakker [1]
Center for Medical Genetics Ghent, Ghent University Hospital, Ghent University, Department of Biomolecular Medicine, Gent-Belgium [1]

An in-frame deletion variant in MAGEL2 (NM_019066.5): c.639_668del & p.(His221_Ala230 del) was detected in two clinically very distinct families. The first family came to attention due to a fetus with hydrops fetalis, lung hypoplasia and fetal akinesia with arthrogryposis. The probands in the second family were a monozygotic twin featuring a Prader-Willi like phenotype with mild intellectual disability, autism spectrum disorder and obesity. Whole exome data (NovaSeq6000 Illumina sequencer) processed with mapper BWA and variant caller GATK HaplotypeCaller revealed a MAGEL2 variant inherited from the father in both families. When

visualizing the data in IGV, the variants were only shown in approximately 1% of the reads, suspicious of a sequencing artefact. The presence of this variant in WES could not be confirmed with a different variant caller (VARDICT), nor could PCR amplicon sequencing on a MiSeq sequencer. Therefore, Sanger sequencing was performed however this was challenging due to the presence of homologous regions in MAGEL2 surrounding the deletion variant. fortunately, PCR and capillary electrophoresis analysis were able to visualize two fragments in the individuals carrying the variant.
As MAGEL2 is an imprinted gene, only individuals inheriting a mutation on the paternal allele will be affected. Extensive segregation analysis in the first family did neither reject the pathogenicity of this variant nor could it be confirmed with certainty. This variant was classified as a variant of unknown clinical significance. Though the clinical symptoms of the probands and segregation results in both families fit within the reported spectrum of MAGEL2-disorders, functional studies might help to further confirm the pathogenicity of this variant. Our study highlights that interesting indel variants can be missed when using short read sequencing.

P1146 - Interstitial 11q deletion in a patient with Sprengel's deformity case report and review of literature

Dhekra Ismail [1], Lilia Kraoua [1], Sylvie Jaillard [2], Hela Bellil [1], Faouzi Maazoul [1], Ridha Mrad [1], Mohamed Nabil Nessib [3], Madiha Trabelsi [1]
Charles Nicolle Hospital, Department of Medical Genetics, Tunis-Tunisia [1] CHU Rennes, Department of Cytogenetics and Cell Biology, Rennes-France [2] Bechir Hamza Children's Hospital, Department of Pediatric Orthopedic Surgery, Tunis-Tunisia [3]

Background: Interstitial chromosome 11 long arm deletions (11q13-q23) represent a rare cytogenetic abnormality characterized by a set of non-specific clinical features including intellectual disability and several malformations without a clear genotype-phenotype correlation.
Methods: Clinical evaluation, standard R-band karyotyping, fluorescence in situ hybridization (FISH) analysis and array Comparative Genomic Hybridization (aCGH) were performed in an 8-year old boy referred because of Sprengel's deformity. We reviewed the literature and attempted to establish a genotype-phenotype correlation.
Results: We described the first case of interstitial 11q deletion identified in a boy with Sprengel's deformity. Phenotype included minor abnormalities such as iris and chorioretinal coloboma, mild motor development delay and a normal intelligence. Karyotype showed a de novo large 11q deletion. FISH analyses confirmed that the deletion is interstitial. Array CGH revealed a deletion of 25.8 Mb encompassing the 11q14.1-q22.3 region. No genotype-phenotype correlation could be established reviewing the 60 previously publ33. clinished cases.
Conclusion: The present case as well as our literature review highlight the clinical heterogeneity and the lack of genotype-phenotype correlation in interstitial 11q deletions. Sprengel's deformity found in our patient might be a new finding in 11q deletions or more probably a fortuitous association.

P1154 - Copy number detection in exome sequencing data for patients with neurodevelopmental disorders an effective approach

Dheedene Annelies [1], D'haenens Erika [1], Delbaere Sarah [1], Rosseel Toon [1], De Bruyne Marieke [1], Callewaert Bert [2], Menten Björn [2]
Ghent University Hospital, Center for Medical Genetics, Ghent-Belgium [1] Ghent University Hospital, Center for Medical Genetics, Department of Biomolecular Medicine, Ghent-Belgium [2]

The use of whole exome sequencing (WES) in the clinic has led to a steep increase in diagnostic yield for patients with neurodevelopmental disorders and congenital anomalies. formerly only single nucleotide variants (SNV) were detected in exome data, but more recently also copy number detection became available. ExomeDepth, a read-depth based analysis tool (Plagnol et al., 2012) was validated for CNV detection on exome data. All reported CNVs were confirmed with an alternative technique (e.g. qPCR, MLPA or CNVseq).
This paper describes the use of ExomeDepth in a diagnostic setting for a period of 20 months. With more than 15 cases in which a pathogenic intragenic CNV was reported, we believe that CNV detection on WES data has the potential to increase diagnostic yield. Several patients harbor a pathogenic deletion in compound heterozygous state with a pathogenic SNV. In one case a bi-allelic pathogenic duplication was observed in a gene associated with an autosomal recessive disorder. In addition in multiple patients a de novo pathogenic deletion was detected, while in one family co-segregation of a deletion of three exons was shown in three affected individuals. Furthermore in two fetal cases a pathogenic CNV was reported that was too small for detection with routine diagnostic molecular karyotyping.
Besides small intragenic CNVs we also detected larger CNVs of several kilobases in some patients for whom (molecular) karyotyping was not yet performed.
Although ExomeDepth is a promising tool, confirmation of all CNVs by an independent technique remains required, as also many false positive calls are made. Given the added value of exome-based CNV detection via ExomeDepth, this tool has now been implemented in our routine exome analysis strategy.

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4. Genomics

P1005 - Improvement of STR based approaches in the analysis of the genetic composition of the Tunisian population and its application in forensic identification.

Asma Attaoui [1], Ali Saad [2]
Minestry of Interior, Scientific and Technical Police, Tunisia-Tunisia [1] Ministry of Health, Farhat Hached Hospital, Tunisia-Tunisia [2]

The more or less homogeneous nature of the Tunisian population (mainly due to consanguinity) poses a huge problem when identifying the actors of crimes, especially in the case of sexual crimes where the analysis of Y haplotypes does not allow the distinction between two individuals.
To remedy these problems and with the aim of reconstructing the identity in criminal and forensic cases in Tunisia, we proposed to use a wide range of genetic markers which will allow us to have a potential of more reliable and meaningful identification.
We expanded our sample to 500 individuals from the Tunisian population collected from crime scenes or taken by forensic doctors (bones from corpses).
We genotyped a large panel of polymorphic markers (STR and SNP) using next-generation sequencing (NGS). We used the forenSeq DNA Signature Prep Kit for the sequencing of these markers (NGS sequencer MiseqFGx). This kit makes it possible to study 23 autosomal markers with a concentration of only 100 pg of genomic DNA. This complementary technology has allowed us to solve certain problems (for example the possibility of analyzing DNA even degraded and/or present in small quantities). The statistical study of the data is provided by the SPSS 26.0 software and the “forenSeq Universal Analysis” software.
Our results show that: (i) autosomal markers D21S11, D12S391, SE33, D2S1338, D18S51 and FGA are the most discriminative (because they have larger and more complex repeat structures compared to single repeat loci) with a diversity attracting allelic forms, (ii) the autosomal markers TH01, DYS391 and TPOX are less discriminating.
To our knowledge, this is the first forensic study , in Tunisia ,to use NGS to determine the distribution of genotypes and frequencies for 23 autosomal STRs. Nevertheless, the search for other discriminative markers present on Chr Y and on Chr X could improve this analysis model. On the other hand, we plan the creation of a complementary database for the investigation of difficult cases specific to the Tunisian population.

P1006 - Case report demonstrating certain pitfalls and challenges in NGS data interpretation

Athina Theodosiou [1], Angelos Alexandrou [1], Ioannis Papaevripidou [1], Ludmila Kousoulidou [1], Constantia Aristidou [2], Nicole Salameh [1], Paola Evangelidou [1], George Tanteles [3], Carolina Sismani [1]
The Cyprus Institute of Neurology and Genetics, Tics and Genomics, Nicosia-Cyprus [1] The Cyprus Institute of Neurology and Genetics, Clinical Genetics Department, Nicosia-Cyprus [2] The Cyprus Institute of Neurology and Genetics, Acropoleos Medical Center, Clinical Genetics Department, Nicosia-Cyprus [3]

Menke-Hennekam syndrome-1(MKHK1) is a congenital disorder characterized by developmental delay, intellectual disability, variable facial characteristics, as well as, feeding difficulties, autistic behaviour, recurrent upper airway infections, hearing impairment, short stature, and microcephaly. It is caused by heterozygous mutations in exon 30 or 31 of the CREB Binding Protein (CREBBP) gene, whereas mutations elsewhere in the CREBBP gene result in Rubinstein-Taybi syndrome-1 (RSTS1), which is phenotypically distinct.
The patient along with his parents was referred for trio-based clinical exome sequencing (CES). The patient is a 21-month-old boy with severe global developmental delay, failure to thrive, arched eyebrows, long lashes and prominent forehead.
CES was performed on Illumina NextSeq 2000 platform using TruSight One sequencing panel. Bioinformatic analysis, annotation and interpretation were performed with the VarSome Clinical platform (version 11.3, hg19).
Sanger sequencing confirmed the CES findings.
A de novo, missense variant was identified and confirmed by Sanger Sequencing, at exon 31 of NM_004380.3 transcript in CREBBP gene (NC_000016.9:3779680A>G), resulting in a cys-to-arg substitution at codon 1790 (Cys1790Arg).
Based on the clinical data, the detected variant was linked to the patient’s phenotype. Even though the same gene is responsible for another phenotypically distinct syndrome (RSTS1), the specific exon, where the variant is located, has differentiated the final diagnosis to MKHK1. This finding highlights the pitfalls and challenges in NGS data interpretation; the importance of a detailed phenotypic description in combination with an in-depth review of all gene-related data available through literature and databases, is determinant to reach an accurate genetic diagnosis.

P1106 - Functional Characterization of a MLH1 missense variant identified in a Tunisian Turcot syndrome patient

Marwa Mahdouani [1], Fahmi Hmila [2], Angela Brieger [3], Stefan Zeuzem [4], Ali Saad [5], Guido Plotz [3], Moez Gribaa [5]
University of Monastir, Higher Institute of Biotechnology of Monastir, Monastir-Tunisia [1] Farhat Hached University Hospital, Department of General and Digestive Surgery, Sousse-Tunisia [2] Goethe University, Biomedical Research Laboratory, Department of Internal Medicine 1, University Hospita, Frankfurt

Am Main-Germany [3] Goethe University, Biomedical Research Laboratory, Department of Internal Medicine 1, University Hospital, Frankfurt Am Main-Germany [4] Farhat Hached University Hospital, Laboratory of Human Cytogenetics, Molecular Genetics and Reproductive Biology, Sousse-Tunisia [5]

Background/Objectives:
Patients with bi-allelic germline mutations in mismatch repair (MMR) genes develop constitutional MMR deficiency (CMMRD), a rare but severe variant of Lynch syndrome. This pathology is distinguished by the presence of early-onset colorectal cancers, lymphomas or leukemias, and brain tumors. Most of the mutations associated with CMMRD are located in the PMS2 gene followed by MSH6, and more rarely MLH1 and MSH2. We identified a homozygous missense alteration in the MLH1 gene (c.1918 C>A; p.Pro640Thr) in one Tunisian patient suspected suffering from Turcot syndrome. A clear classification of pathogenicity for this variant was not available, consequently diagnosis, predictive testing and targeted surveillance in affected family members was impossible.
Methods:
We performed functional laboratory testing, for the first time in Tunisia, using a system testing stability as well as catalytic activity that includes clinically validated reference variants.
Results:
The variant p.Pro640Thr was found to be non-functional due to severe defects in protein stability and catalytic activity. Analysis of residue conservation and of the structural roles of the substituted residues corroborated these findings. In conjunction with the available clinical data, this variant fulfill classification criteria for class 4 “likely pathogenic”.
Conclusion:
These findings will improve genetic counselling and clinical management of affected Tunisian family and other carriers of this genetic variant worldwide.

P1113 - Molecular characterization of Porokeratosis in Tunisian patients

Haifa El Mabrouk [1], Dong-Lin Li [2], Lobna Bousoffara [3], Nadia Ghariani [3], Mohamed Denguezli [3], Ali Saad [4], Cristina Has [2], Dorra H'mida [4]
University of Monastiir, Higher Institute of Biotechnology of Monastir, Monastir-Tunisia [1] Freiburg University, Department of Molecular Dermatology, Freiburg-Germany [2] Farhat Hached University Hospital of Sousse, Department of Dermatology, Sousse-Tunisia [3] Farhat Hached University Hospital of Sousse, Laboratory of Human Cytogenetics, Molecular Genetics, and Reproductive Biology, Sousse-Tunisia [4]

Background/Objectives: Porokeratosis (PK) is a group of rare acquired or inherited clonal keratinization disorders characterized by annular or linear hyperkeratotic plaques with central atrophy and raised borders. They share a common histological feature: the cornoid lamella. The mevalonate pathway's genes are implicated in the pathogenesis of this genodermatosis. To date, the underlying molecular mechanisms remain misunderstood.
Methods: We sought to uncover the molecular landscape of 8 Tunisian patients' PK profiles using Sanger sequencing, RT-PCR and in silico analysis.
Results: In this study, we investigated a Tunisian family with autosomal recessive non-inflammatory PK in whom we reported an important clinical heterogeneity with a simultaneous presence of three clinical forms of PK in some individuals. Two PMVK variants, nonsense and synonymous, have been identified in affected individuals and unaffected relatives.
Conclusion: Our molecular investigations suggest the incrimination of the haploinsufficiency of PMVK protein due to the degradation of nonsense mRNA mutant by the NMD pathway. We suggest that the PK profiles of our Tunisian patients are a result of the germline nonsense mutation and a postzygotic second-hit mutation in the skin lesions.

P1116 - Telomere dysfunction leads to chromosomal aberrations in patients with disorders of sexual development

Haifaou Younoussa[1-2], Bruno Colicchio[3], Ahmed Said[4], Steffen Junker[5], Andreas Plesch[6], Leonhard Heidingsfelder[6], Eric Robinet[7], Ndeye Rama Diagne[8], Alain Dieterlen[3], Philippe Voisin[1], Patrice Carde[9], Eric Jeandidier[10], Mamadou Soumboundou[8], Radhia M’Kacher[1], Macoura Gadji[2]
[1]Cell Environment, DNA damage R&D, Evry, France, [2]Service of Biological Hematology & Oncology-Hematology (BHOH), Faculty of Medicine, Pharmacy and Odonto-Stomatology, Cheikh Anta Diop University of Dakar (UCAD), National Centre of Blood Transfusion (NCBT), Dakar, Senegal
[3]IRIMAS, Institut de Recherche en Informatique, Mathématiques, Automatique et Signal, Université de Haute-Alsace, Mulhouse 68093 France, [4]Quantoom Biosciences, Evry, France, [5]Institute of Biomedicine, University of Aarhus, Aarhus DK-8000, Denmark, [6]MetaSystems GmbH, D-68804 Altlussheim, Germany, [7]Lymphobank, Besancon, France, [8]Medical Diagnostic Laboratory, Diamniadjo Hospital, UFR-Santé de Thiès, Iba Der THIAM University of Thies, Senegal, [9]Department of Hematology, Gustave Roussy Cancer Campus, Villejuif, France, [10]Service de génétique Groupe Hospitalier de la Région de Mulhouse et Sud Alsace Mulhouse, France

Disorders of sex development (DSD) are congenital anomalies characterized by atypical gonadal and anatomical sex development resulting in fertility disorders and hormonal insufficiency. Here, we investigate cytogenetic profiles (*i.e.* telomere and

chromosomal aberrations) as well as genomic variations in Senegalese DSD patients.
Materials and methods:
Peripheral blood lymphocytes (PBL) were isolated from 35 DSD patients (mean age: 9 years; range 0-18 years) from two hospital centers in Dakar, Senegal. PBLs from one hundred healthy donors with similar age were used as a controls as well as twenty pathological prenatal samples and 50 cord blood from healthy women. For the analyses we employed conventional cytogenetics, telomere and centromere (TC) staining followed by multiplex FISH (M-FISH), and FISH with gene-specific probes such as *SRY*. Genomic analysis of genes involved in gonadal differentiation and DSD were performed using PCR amplification and Sanger sequencing.
Results:
Cytogenetic analysis identified 18 male and 14 female patients, 2 patients with Turner syndrome and one patient with Klinefelter syndrome. Additional structural chromosome aberrations were detected in 22% of the patients (8/35). Telomere analysis revealed reduction of mean telomere lengths of DSD patients compared to those of healthy donors or cord blood. Nevertheless, telomere lengths of DSD were similar to telomere lengths of pathological prenatal samples. This reduction of telomere length was associated with the increase of telomere aberrations (telomere loss and the formation of telomere doublets). Genomic analysis revealed mutations and deletions in the genes encoding 5 Alpha-reductase-2 (SRD5A2) and CYP 21A2, respectively, and in the *androgen receptor (AR)* gene that is necessary for normal male sexual development.
Conclusion:
To the best of our knowledge this study is the first to demonstrate a correlation between telomere dysfunction and DSD thus underscoring the relevance of implementing telomere analysis in prenatal tests as well as in the proven genetic DSD disorders. Genomic analyses will be pursued to identify additional new biomarkers of DSD that will be easy to employ in developing countries.
Key words: DSD, chromosomal aberrations, telomere, genomic alterations

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P1138 - Switching on sex Genetic findings in a Tunisian cohort with Disorders of sexual development (DSD)

Khouloud Rjiba [1], Imen Hadj Hamida [1], Ghada Saad [2], Bochra Khadija [1], Afef Jelloul [1], Anu Bashamboo [3], Kenneth Mcelreavey [3], Soumaya Mougou [1]
Farhat Hached University Teaching Hospita, Laboratory of Human Cytogenetics, Molecular Genetics and Biology of Human Reproduction, Farhat Hached University Teaching Hospital., Sousse-Tunisia [1] Farhat Hached University Teaching Hospita, Department of Endocrinology, L, Sousse-Tunisia [2] Institut Pasteur, Human Developmental Genetics Unit, Cnrs Umr 3738,, Paris-France [3]

Introduction: Disorders of sex determination/differentiation (DSD) comprise a heterogeneous group of congenital conditions in which chromosomal, gonadal or anatomical sex is discordant. In recent years, a number of new candidate genes have been identified to be associated with DSD and the diagnostic yield has been remarkable.

Patients and Methods: The aim of our study is to identify the genetic etiologies of DSD and offer knowledge-based genetic counseling and a genetic strategy for exploration. Our cohort includes 143 patients with different clinical picture and were explored with a dual approach: cytogenetic and molecular.
Results and discussion: The genetic etiology was assigned in 69.4% (93/134) of cases. The cytogenetic approach was able to identify chromosomal rearrangements, including sex chromosome abnormalities, in 71% (66/93) of the explained cases, whereas molecular analysis allowed identifying a genetic etiology in 29% of explained cases. Exome sequencing was able to attribute a genetic cause in 26 out of 66 tested patients by this technique. Novel and rare pathogenic variants in known genes involved in developmental regulatory pathways and sexual differentiation (AR, SRD5A2, LHCGR, MCM8, FIGLA, CHD7, FGFR1 and GNRHR) were identified thus expanding the phenotypic and genetic spectrum within these genes. Pathogenic variants in newly described genes such as ZNRF3, SOX8 and HHAT were identified. These results reinforce the integration of the ZNRF3, SOX8 and HHAT genes in Wnt regulation pathway, the FOXL2 signaling pathway and post-translational modifications (palmitoylation), respectively. Interestingly, a homozygous variation in a gene (SART3), not known to be involved in DSD, coding for a novel spliceosomopathy affecting neuronal and testicular development was identified.
Conclusion:
Our data enrich the network of the proteins involved in DSD with a heterogeneous and pleiotropic phenotypic spectrum. Furthermore, our study emphasizes the usefulness of both cytogenetic approaches as well as exome sequencing to make an accurate genetic diagnosis for genetic counseling and knowledge-based management of patients with DSD.

P1148 - Somatic and Germline Variants in Ovarian Cancer Patients from a Unique Geographically Isolated Population A Comprehensive Molecular Analysis

Federica Cannas [1], Alessia Mascia [2], Stefano Mocci [1], Stefania Tranquilli [1], Chiara Cocco [1], Michela Lorrai [1], Caterina Mereu [1], Celeste Sanna [1], Laura Ghelfi Zoboli [1], Giancarlo Mancuso [1], Alessia Bucca [1], Federico Marongiu [1], Giulia Nutile [1], Francesco Lai [2], Michela Mura [1], Ludovica Martorana [3], Roberta Murru [3], Nicola Orrù [3], Erika Giuressi [3], Sabrina Giglio [1]

University of Cagliari, Medical Sciences and Public Health, Cagliari-Italy [1] University of Cagliari, Biomedical Sciences, Cagliari-Italy [2] Assl Cagliari, Medical Genetics Unit, R. Binaghi Hospital, Cagliari-Italy [3]

Ovarian cancer (OC) is a heterogeneous group of tumors with different cell origins and genomic features. Although BRCA1 and BRCA2 pathogenetic variants are observed in about 25-30% of ovarian cancer cases, the frequency of these and other variants in geographically and genetically isolated populations, such as Sardinia, is poorly characterized.
This study explored the molecular landscape of germline and somatic variants in Sardinian women with ovarian cancer, which is known to exhibit a unique genetic background.
We analyzed tissue and blood samples from 79 Sardinian patients with epithelial ovarian cancer using a multigene panel, WES and SNP-array analysis.
Our results show that BRCA1 and BRCA2 somatic variants were present in 13% (11/79) and 7.5% (6/79) of the patients, respectively.
of our patients, 32 with high-grade serous carcinoma and genomic instability exclusively had variants in the TP53 gene in tumor tissue. So, pathogenic variants in the TP53 gene were found in 78% (62/79) of the patients. Moreover, 30 cases carried additional variants in less-common OC-associated genes: ATM, CHEK2, PTEN, PIK3CA, FBN1, ARID1A, BUB1, CDHR1, MSHS2, MASH6, MLH1, STK11
Our findings highlight the importance of expanding the clinical search for tumor predisposition genes in regions with genetic isolation, where minor genetic variability and greater cancer predisposition may exist. This will help improve strategies for future therapeutic optimization and identify additional candidate genes for treatment in ovarian cancer, especially in populations like Sardinia, where the frequency of BRCA1 and BRCA2 mutations is lower than in the rest of Italy.

P1154 - Copy number detection in exome sequencing data for patients with neurodevelopmental disorders an effective approach

Dheedene Annelies [1], D'Haenens Erika [1], Delbaere Sarah [1], Rosseel Toon [1], De Bruyne Marieke [1], Callewaert Bert [2], Menten Björn [2]
Ghent University Hospital, Center for Medical Genetics, Ghent-Belgium [1] Ghent University Hospital, Center for Medical Genetics, Department of Biomolecular Medicine, Ghent-Belgium [2]

The use of whole exome sequencing (WES) in the clinic has led to a steep increase in diagnostic yield for patients with neurodevelopmental disorders and congenital anomalies. formerly only single nucleotide variants (SNV) were detected in exome data, but more recently also copy number detection became available. ExomeDepth, a read-depth based analysis tool (Plagnol et al., 2012) was validated for CNV detection on exome data. All reported CNVs were confirmed with an alternative technique (e.g. qPCR, MLPA or CNVseq).
This paper describes the use of ExomeDepth in a diagnostic setting for a period of 20 months. With more than 15 cases in which a pathogenic intragenic CNV was reported, we believe that CNV detection on WES data has the potential to increase diagnostic yield. Several patients harbor a pathogenic deletion in compound heterozygous state with a pathogenic SNV. In one case a bi-allelic pathogenic duplication was observed in a gene associated with an autosomal recessive disorder. In addition in multiple patients a de novo pathogenic deletion was detected, while in one family co-segregation of a deletion of three exons was shown in three affected individuals. Furthermore in two fetal cases a pathogenic CNV was reported that was too small for detection with routine diagnostic molecular karyotyping.
Besides small intragenic CNVs we also detected larger CNVs of several kilobases in some patients for whom (molecular) karyotyping was not yet performed.
Although ExomeDepth is a promising tool, confirmation of all CNVs by an independent technique remains required, as also many false positive calls are made. Given the added value of exome-based CNV detection via ExomeDepth, this tool has now been implemented in our routine exome analysis strategy.

P1157 - Breastfeeding promotes persistence of the mother's chimeric cells in their offspring

Boris Dmitrenko [1], *Vincent Gatinois* [2], Morgana D'Ottavi [1], Ahmed El Mouatani [2], Pauline Bouret [2], Ségolène Debiesse [1], Michel Mondain [3], Mohammed Akkari [3], Maxime Dallemagne [3], Anouck Schneider [2], Franck Pellestor [2], Philippe Vande Perre [1], Jean-pierre Molès [1]
Inserm, University of Montpellier, University of Antilles, Pathogenesis and Control of Chronic And Emerging Infections, Montpellier-France[1] CHU Montpellier, University of Montpellier, Research Hospital Plateform Chromostem, Montpellier-France [2] CHU Montpellier, University of Montpellier, Department of ENT and Head and Neck Surgery, Montpellier-France [3]

The mechanisms underlying the benefit of breastmilk for child health are still incompletely elucidated: physiological processes may be implicated such as those involving maternal cell transfers to the offspring, and those that sustain maternal cell retention throughout infant life. Breastmilk contains maternal cells primarily consisting of epithelial cells but also of myeloid and lymphoid cells up to 6% of stem cells.
In the present study, we aimed to identify female XX cells of maternal origin in secondary lymphoid tissues from young males by coupling a cytogenetic sex chromosomes Fluorescent in situ Hybridization

(FISH) with an optimized automated microscopic quantification approach. Our secondary objectives were: (i) to give a quantitative analysis of the microchimeric cell counts and (ii) to investigate possible associations with infant feeding modalities in early infancy.
We identified a total of 48 female nuclei for a total of 1,166,308 scanned nuclei. Female cells were identified in 57.9% (22/38) of patients. Final multivariate analyses for factors associated with female cell density showed that, when accounting for whether the child was previously breastfed or not, female cell density was associated with sibling rank. In addition, older mother's age was inversely associated with female cell density.
The microchimeric cell transfer from mother to child during breastfeeding was strongly suggested although not demonstrated. However, the persistence of microchimeric cells of maternal origin was associated with previous breastfeeding, suggesting tolerogenic properties of these cells.

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5. Prenatal Diagnosis

P1013 - One Laboratory's Experience – Concordance between NIPT Karyotyping FISH and Prenatal CMA for Diagnosing Chromosomal Anomalies

Xin Yi Boo [1], Mui Li Tan [1], Lee Tian Toh [1], Hui Yi Yon [1], Sharon Ai Chien Ling [1], Rachel Tze Yoong Phua [1], Hwei Meeng Angeline Lai [2], Min Hwee Yong [1]
Kk Women's and Children's Hospital, Cytogenetics Laboratory, Department of Pathology and Laboratory Medicine, Singapore-Singapore [1] KK Women's and Children's Hospital, Genetics Service, Paediatrics, Department of Medicine, Singapore-Singapore [2]

In this study, we aim to compare the results between non-invasive prenatal testing (NIPT), karyotyping, fluorescence in-situ hybridization (FISH) and prenatal chromosomal micro-array (CMA).
The Cytogenetics laboratory in KK Women's and Children's Hospital (KKH) has received 119 samples (amniotic fluid, blood, chorionic villi and product of conception) from year 2017 to 2021, with the clinical indication of "high-risk", "low-risk (but with other ultrasound indications)", "inconclusive" or "no" NIPT results.
45 out of 77 samples (58.4%) with high-risk NIPT results were concordant with karyotyping or CMA results. Within these 77 samples, the sensitivity of NIPT in detecting Trisomy 13, 18, 21 and sex chromosome aneuploidies (SCA) are at 37.5%, 50%, 87% and 50% respectively. All five NIPT samples, which showed high-risk for microdeletion 22q11.2, were tested negative by FISH or CMA. 15 out of 31 cases (48.4%) of SCA showed non-concordance and this could be due to the presence of a vanishing twin or confined placental mosaicism.
Among the 25 low risk NIPT samples, one was found to be false negative for Trisomy 21 after karyotyping and two had additional findings through CMA (variant of uncertain clinical significance on chromosomes 2 and 9).
Six samples with no NIPT results and 11 samples with inconclusive SCA NIPT results were normal by karyotyping or CMA.
Based on our data, we can conclude that NIPT is effective in detecting Trisomy 21, but for other chromosomal abnormalities, NIPT tends to give false positive results. Hence, karyotyping, FISH or CMA should be recommended to confirm high-risk NIPT results for these abnormalities as the sensitivity and specificity for them are low due to lower positive predictive value. Karyotyping and CMA methods are also useful in detecting mosaic and unbalanced translocation cases, providing additional information to the clinicians for the counseling of patients.

P1029 - The ever changing face of Cytogenetics Units Use and contribution of Whole Exome Sequencing in prenatal diagnosis.

Celine Dupont [1], Jonathan Rosenblatt [2], Myriam Rachid [1], Brian Sperelakis Beedham [1], Olivier Sibony [2], Sabatini Jeyarajah [1], Cecile Le Long [1], Elka Kurtova [1], Laurence Perrin [3], Yline Capri [3], Ilyas Challet [3], Emilie Serrano [3], Marjorie Delepine [3], Claire Loraschi [2], Severine Drunat [4], Cedric Vignal [4], Jonathan Levy [1], Anne-Claude Tabet [1]
Robert Debré Hospital Ap-hp, Cytogenetics Unit, Genetics Department, Paris-France [1] Robert Debré Hospital Ap-hp, Obstetrics, Paris-France [2] Robert Debré Hospital Ap-hp, Clinical Genetics Unit, Genetics Department, Paris-France [3] Robert Debré Hospital Ap-hp, Molecular Genetics Unit, Genetics Department, Paris-France [4]

On a large number of high-risk pregnancies, we have acquired a solid experience in prenatal cytogenetics testing, including a better understanding of which chromosomal anomalies to expect for each ultrasound findings (study under revision). First reported in research studies, NGS has become essential in the diagnosis of genetic abnormalities in patients with suggestive signs. In the cytogenetics unit, we have implemented this technology in our diagnosis strategy. After review from the prenatal diagnosis center, whole exome sequencing (WES) was performed for some fetuses when standard cytogenetics analysis including CMA was normal.
We report the results of 35 WES analyzed in trio or quartet. A diagnosis was reached in 7 cases, through detection of pathogenic or likely pathogenic variants (7/33 : 21.2%) in NRAS, NEU1, TNNI2, FGFR3, CPLANE1, PIEZO1 and NOTCH2 genes. We will describe these variants, the majority of which have not been previously reported. The presence of these variants was confirmed by sanger sequencing. In most cases, we were able to provide the results to the

patients before the end of the pregnancy. Sequencing failed however in two cases (2/35: 5.71%)
Diagnostic yield of prenatal WES is known to vary greatly according to indication but our first results are promising. The WES is now part of our diagnostic tools and strategy for high-risk pregnancies. This efficient technology has already allowed us to adapt our genetic counseling towards some types of malformations, sometimes even reassuring couples.

P1031 - A role of cytogenetic methods in prenatal diagnostics (case study)

Helena Peková [1], Magda Plachá [1], Marie Trková [1], Jiří Horáček [1], Kateřina Sobolová [2]
Gennet, Cytogenetic Laboratory, Praha 7-Czechia [1]
Gennet, Clinical Genetic, Praha 7-Czechia [2]

Cytogenetic analysis still plays an important role in clinical diagnostics. We present here a recent case of prenatal diagnostics in a pregnancy of a 34 yrs old women with a positive first trimester biochemical screening and negative a non-invasive prenatal test for chromosomal defects in the fetus (PRENA SCAN). An amniocentesis was performed in her 18th gestational week and consequent SNP array analysis detected a partial duplication (12.5 Mb) of chromosome 11 in 11p15.5-15.3 region. The duplication was confirmed by karyotyping and FISH method using locus specific probes. Both methods showed that the duplicated part of chromosome 11 was translocated on satellites of chromosome 14. Clinically, this microduplication overlaps with 11p15.4 microduplication syndrome and affects several imprinted genes. After confirming parental origin of the duplicated chromosomal segment of the fetus parents decided to terminate the pregnancy.

P1034 - Prenatal diagnosis of Beckwith Wiedemann syndrome a case report

Cristina Ferreira [1], Ana Tarelho [1], Bárbara Marques [1], Sílvia Serafim [1], Sónia Pedro [1], Carla Granja [2], Rodrigo Mata [2], Ana Teresa Martins [3], Inês Carvalho [3], Hildeberto Correia [1]
Instituto Nacional De Saúde Doutor Ricardo Jorge, I.p., Departamento De Genética Humana, Unidade De Citogenética, Lisboa-Portugal [1] Hospital De Portimão, Centro Hospitalar Do Algarve, Serviço De Obstetrícia / Diagnóstico Pré-natal, Portimão-Portugal [2] Maternidade Dr. Alfredo Da Costa, Centro Hospitalar De Lisboa Central, Epe, Centro De Diagnóstico Pré-natal, Lisboa-Portugal [3]

Beckwith-Wiedemann syndrome (BWS) is the most common overgrowth disease. Phenotypically and genetically heterogeneous, it is linked with epigenetic/genetic aberrations on 11p15.4p15.5 chromosome region and the majority of cases are diagnosed after birth with prenatal diagnosis being difficult and depending on the identification of specific ultrasound (US) anomalies. Here we present a case of a fetus from a healthy 21-year-old primiparous woman, with a low risk in the first trimester aneuploidy screening and bilateral increase in renal volume detected in the second trimester US. An amniotic fluid (AF) sample was collected at 24 weeks. Rapid aneuploidy diagnosis by QF-PCR and sequencing of a multigene panel for renal dysplasia were performed with a normal result for common aneuploidies and detection of a variant with uncertain significance, respectively. The pregnant women was referred to a differentiated prenatal diagnosis center and a detailed US at 30 weeks and 3 days showed multiple features suggestive of BWS: macroglossia, weight estimation at P100, significantly enlarged kidneys without cortico-medullary differentiation, hepatomegaly, prenasal and cervical subcutaneous thickening, and suspected cardiomegaly without structural heart disease. The methylation pattern study of 11p15 region by MS-MLPA revealed hypermethylation of H19/IGF2 differentially methylated region (DMR), confirming the Beckwith-Wiedemann diagnosis.
In contrast to postnatal cases that can be diagnosed by phenotypic scoring, prenatal diagnosis of BWS is relatively challenging because some common features cannot be detected by US and other features appear only after 30 weeks of gestation and may be missed on second-trimester morphological ultrasound. Omphalocele is the most common prenatal phenotypic BWS-associated feature, and is the first and most easily identified in prenatal screening, but there are other features that may suggest BWS such as macroglossia, macrosomia, placental mesenchymal dysplasia, polyhydramnios, and visceromegaly. Routine molecular testing of all fetuses with one or more prenatal BWS-associated features is of utmost importance, as early diagnosis is significantly beneficial for prenatal counselling, perinatal management allowing for better birth planning and postnatal care for neonatal hypoglycemia, respiratory distress, and risk of malignancy.

P1042 - Inherited unbalanced reciprocal translocation with 18p tetrasomy and 9q34.3 trisomy in a foetus revealed by cell free foetal DNA (cffDNA) testing cytogenetic and cytogenomic characterization in prenatal diagnosis

Luigia De Falco [1], Carmela Ardisia [2], Giovanni Savarese [1], Raffaella Ruggiero [1], Pasquale Savarese [1], Teresa Suero [1], Antonella Di Carlo [1], Mariasole Bruno [1], Nadia Petrillo [1], Rosa Castiello [1], Monica Ianniello [1], Maria Rosaria Fantuz [1], Maria Simonetti [1], Davide Cino [1], Maria Barbato [1], Alessio Mori [1], Giuseppina Vitiello [3], Antonio Fico [1]
Ames, Centro Polidiagnostico Strumentale, Srl, Medical Genetics, Naples-Italy [1] S. Maria Della Misericordia Hospital, Medical Genetics, Perugia-Italy [2] Federico Ii University Hospital, Molecular Medicine and Medical Biotechnologies, Naples-Italy [3]

Balanced reciprocal translocations are common human genetic abnormalities that involve the exchange of the segments between different chromosomes and have an approximately 50-80% chance of generating an embryo with chromosomal abnormalities. Here we describe a case with unusual structural chromosome rearrangements evidenced by non-invasive prenatal testing (NIPT).

A 37-year-old Caucasian woman, at 13+5 weeks of gestation, carrier of a reciprocal and balanced translocation 46, XX, t (9;18) (q34; q11.2)) and a history of polyabortion came to our attention to undergo the NIPT test, considering the risk of invasive prenatal diagnosis. NIPT, carried out using VeriSeq™ NIPT Solution v2 assay (Illumina Inc.), showed a high risk for chromosome 18 aneuploidy. Amniocentesis was carried out at 16+0 weeks. A second trimester ultrasound scan showed no malformations. Quantitative fluorescence polymerase chain reaction (QF-PCR) (Devyser Compactv3), for the trisomies 21, 13, 18 and sex chromosome aneuploidies showed partial trisomy of short arm of chromosome 18.Cytogenetic analysis performed on the flask cultured amniocytes indicated the 48,XX,+2mar on 50 metaphases. Single Nucleotide Polymorphism (SNP) array analysis (HumanCyto SNP-12v12.1 kit) showed a 15.3 Mb duplication of the chromosome 18p (arr[hg19] 18p11.32-p11.21(12,842-15,303,932) x4) consistent with tetrasomy 18p and a 926 kbp duplication of chromosome 9q (arr[GRCh37] 9q34.3(140,118,286-141,044,489)x3)consistent with trisomy 9q. After genetic counseling, the couple decided to terminate the pregnancy at 19+4 weeks.

The evidence of such partial aneuploidies suggests that different mutational events may be possible, both at segregation and post-meiotic time. This case confirms the high sensitivity of NIPT, based on genome-wide massive parallel sequencing for common aneuploidies, as well as for the detection of sub-chromosomal abnormalities. In addition, the use of cytogenetics, cytogenomics and molecular biology techniques, in synergy, help to characterize and confirm the positive NIPT test results.

P1066 – Anticipation of Sex Discrepancies in non invasive Prenatal Testing Due To Maternal Genetic Abnormalities

Nuria Balaguer [1], José Antonio Martínez-conejero [2], Emilia Mateu-brull [1], Ana Cerveró [2], Miguel Milán [1]
Igenomix Spain Lab S.l.u., Prenatal Diagnosis, Paterna-Spain [1] Igenomix Spain Lab S.l.u., Pre-implantational Genetic Diagnosis, Paterna-Spain [2]

Sex discordance between cell-free DNA (cfDNA) testing and ultrasound examination is rare, but causes patient discomfort and uncertainty. Here we present two clinical cases where closer examination of the raw sequencing data allowed us to anticipate possible discrepancies caused by the insertion of Y-chromosome regions into the maternal genome.

Illumina's Veriseq NIPT Solution v2 and a proprietary bioinformatics pipeline were used to analyse the cfDNA in the maternal bloodstream. Paired-end sequencing data were aligned to the reference genome (hg19). Non-duplicated aligned reads were aggregated into 50 kb bins. The corresponding bin counts were adjusted for GC bias and finally aggregated per 5Mb windows. Z-scores were calculated for autosomes, sex chromosomes and 5 Mb bins.

The two clinical cases were classified as low-risk male fetuses according to the main statistics (case A: NCV_Y= 40.743, NCV_X= -0.345, fetal fraction (FF)=11%; case B: NCV_Y=40.553, NCV_X= 0.261, FF=5.1%). The Y-chromosome-based FF (FFy) was significantly lower than the default FF estimate (FFy=2% in both cases). Plots of X and Y chromosome z-scores for each 5 Mb bin according to genomic position allowed us to identify bins with z-scores significantly higher than those expected for any pregnancy with a male fetus. The genomic coordinates of these bins (case A: ChrY:7000000-7500000; case B: ChrY:6500000-7000000) overlapped with the protein kinase Y-linked (PRKY) and amelogenin (AMELY) genes, respectively. Amplification of these regions in the white blood cell fractions of each maternal plasma confirmed the presence of Y-chromosome insertions in both maternal genomes.

Here, we describe a new source of discrepancy to add to those already known and described. These findings provide a way to improve current bioinformatics pipelines to identify possible maternal perturbations and exclude them from the classification algorithms used for aneuploidy and sex calls.

P1083 - Prenatal diagnosis of a 15q24.1 microdeletion in a fetus with cerebral and urogenital abnormalities

Anaik Previdi [1], Penelope Jordan [1], Charles Egloff [2], Aurelie Coussement [1], Samira Ahmed-Elie [1], Laure Tudal [2], Olivier Picone [2], Jean-Michel Dupont [1]
Aphp.centre-université Paris Cité, Site Hôpital Cochin, Service de Médecine Génomique des Maladies de Système et d'Organe, Paris-France [1] Aphp.nord-université Paris Cité, Site Hôpital Louis Mourier, Service de Gynécologie Obstétrique, Colombes-France [2]

We report here the third known case of prenatal identification of 15q24.1 microdeletion. The fetus displayed cerebral abnormalities including hydrocephaly with macrocephaly, cerebellum hypoplasia with vermis hypoplasia, and rhombencephalosynapsis. Ultrasound scans also revealed right kidney agenesis with left kidney duplication and micropenis.

15q24.1 microdeletion syndrome is a recently described condition that often results from non-allelic homologous recombination between low copy repeats (LCRs). Typical clinical features of this syndrome usually include pre and post-natal growth retardation, facial dysmorphism, developmental delay and

intellectual disability. While nonspecific urogenital, skeletal, and digit abnormalities may also be present, other congenital malformations are less frequent, which makes this syndrome difficult to identify before birth. As a result, there have been only two cases reported prenatally, which complicates the genotype-phenotype correlation.
We describe a new case with multiple congenital malformations that allowed for a diagnosis at 26 weeks of amenorrhea. Cerebral malformations are typically nonspecific, but microcephaly appears to be the most frequent in postnatal cases. Our case is the first reported with a frank cerebellar involvement (rhombencephalosynapsis, hypoplasia of the vermis) and macrocephaly due to severe hydrocephaly.
We compared our case with previously published cases in the literature to further delineate the prenatal clinical features and the candidate genes involved in the phenotype.

P1093 - Expression of the syncytin 1 and syncytin 2 genes in the trophoblastic tissue of the early pregnancy losses with normal and abnormal karyotypes

Esra Tug [1], Meral Yirmibes Karaoguz [1], Tuncay Nas [2]
Gazi University, Faculty of Medicine, Medical Genetics, Ankara-Turkiye [1] Gazi University, Faculty of Medicine, Obstetrics and Gynaecology, Ankara-Turkiye [2]

The products of Syncytin-1 and syncytin-2 which are endogenous retroviral genes play a major role in fusion process involved in syncytialization of trophoblasts in placental development and maintenance of healthy pregnancy. In the presence of cytogenetically abnormal karyotype, effect of syncytin expressions on syncytialization process and in occured to spontaneous abortions is not clear. To reveal this, we investigated in syncytin-1 and syncytin-2 expressions of chromosomally abnormal and normal trophoblastic tissues and also discussed impact of these on spontaneous abortion. From cultivation, harvesting, banding, and analyses were performed to trophoblastic cells, and classified according to presence of abnormality and normal XY constitution. Samples were grouped as trisomy 16 (n=10), triploidy (n=9), monosomy X (n=9), trisomy 21 (n=5), trisomy 7 (n=3) and XY karyotype (n=20). Frozen trophoblastic cells were used for RNA isolation, and proceeded to determination of expressions of syncytin-1 and syncytin-2 by single-step RT-PCR. From cDNAs obtaining in same PCR stages, sequence analyses of the syncytin-1 and syncytin-2 were performed. Between expressions of syncytin-1 and syncytin-2 were statistically difference in the patients and controls. There was a difference between trisomy 7 and other patient groups and controls, regarding to expression of syncytin-1. Numerous mutations were detected in sequence analysis of expressed region of syncytin-1 and syncytin-2. In the patient and control groups, mutation rate was higher in syncytin-1 than in syncytin-2. This study indicate that expression of syncytin-2 could be altered in presence of chromosomally abnormal trophoblastic tissues and could lead to loss of pregnancy due to insufficient syncytialization. Current research has value for further studies covering mechanisms of trophoblast cell fusion, and syncytiotrophoblast regeneration, and thus the pathophysiology of human placental development in presence of genomic anomaly.

P1127 - Have the objectives been met in the screening for aneuploidies in TPNI

Rosa Maria Lobo [1], David Arrabak [2], Manuel Gonzalez [3], Sogicyl Prenatal [4]
Hospital Universitario Rio Hortega, Genetic, Valladolid-Spain [1] Ospital Universitario Rio Hortega, Genetic, Valladolid-Spain [2] Ospital Universitario Rio Hortega, Investigation, Valladolid-Spain [3] Hospital Universitario De Burgos, Regional Society, Burgos-Spain [4]

The initial objectives were two fold: increase detection rates and decrease invasive tests.
The form of implementation was within the framework of a contingent screening with a cut-off value of less than 1/50 and greater than 1/1000
The results reported included aneuploidies of chromosomes 13, 18, 21, X and Y, only, from 2020 expanded prenatal screening was reported in the case of a non-viable fetus.
After four full years, from 2018 to 2021 we proceed to review the results to see if the initial objectives have been achieved.
MATERIAL AND METHODS
All cases with combined risk between 1/50 and 1/1000 only have been selected.
In the Autonomous Community of Castilla y León, this advanced test is performed sequentially to classic screening (maternal age + MoM free fraction β-hCG and PAPP-A + NT) that classifies pregnant women into three risk groups: high risk (<1 / 100, direct invasive test), low risk (≥1000) and intermediate risk (1 / 100-1 / 1000, study in circulating fetal DNA)
RESULTS
5956 tests have been requested with results
The reduction of invasive tests has been 40.5% in 2018, 52.1% in 2019, 61% in 2020 and 84% in 2021.
The increase in detection ratios when performing NIPT from 1/270 to 1/1000 has led to the detection of an additional 50% of fetuses with chromosomal abnormalities of chromosomes 13, 18, 21, X and Y.
CONCLUSIONS
The results obtained in these 4 years conclude that the initial objectives set have been met, the detection rates have increased by 50% compared to the classical screening carried out in which, from a cutoff of 1/270, no further actions were carried out until 20 week morphology ultrasound

The reduction in invasive tests has also been achieved, with a notable decrease of 84% during the year 2021. The only adverse event that we have had has been the non-prenatal detection of a fetus that turned out to have trisomy 21 with a risk in the classic screening of 1/1933. This makes us consider whether it would be possible in economic terms to raise the cut-off value to 1/2000 or 1/3000.

P1133 – Deletion of Exon 16 of the COL2A1 Gene in Prenatal Spondylo-Epiphyseal Dysplasia

Maria Rosa Martorell Riera [1], Laura Torres-Prats [2], Sara Martinez [2], Fernando Santos-Simarro [1], Angeles Pérez-Granero [1], Damià Heine [1], Iciar Martínez [1]
Hospital Universitario Son Espases, Unidad de Diagnóstico Molecular Y Genética Clínica, Palma de Mallorca-Spain [1] Hospital Can Misses, Servicio Ginecologia, Ibiza-Spain [2]

Introduction
Congenital spondyloepiphyseal dysplasia (SEDC) is a disease of low prevalence and patients present with short stature at birth with flattened vertebrae, with absence of ossification in pubic bones, absence or reduction of the sacral and cervical vertebrae.
We present a prenatal case of SEDC detected at 19 weeks of gestation after obstetric evaluation.
Material and methods
The fetus of a 31-year-old pregnant woman showed a Clubfeet, incomplete ossification of the vertebral bones, short long bones and micrognatia. The couple decided to legally terminate the pregnancy. A post-mortem babygram and clinical exome was requested.
Results
QF-PCR and CGH-arrays were normal and determined the male foetus.
Trio analysis of the whole exome sequence and analysis of genes related to skeletal bone dysplasias suggested a complete de novo heterozygous deletion of exon 16 in the COL2A1 gene, NM_001844 Hg19 12q13.11 (chr12:48386649-48386724)x1. This deletion was later confirmed by MLPA (Multiplex Ligation-dependent Probe-Amplification) with a MRC-Holland P214 Salsa.
Discussion
Missense variants are the most frequent mutations in the COL2A1 gene, however, in our case we detected a deletion affecting the entire exon 16. Previous reports of COL2A1 delections are restricted to Barat-Houari et al. 2016, who detected a large deletion (exons 41-46) in a patient with achondrogenesis. We have not found any other case with an entire exon 16 deletion in the current databases, LOVD, Decipher and Clin Var.
Routine prenatal diagnostic techniques such as low-coverage CGH-arrays usually do not cover small deletions. In cases of skeletal dysplasia it may be of interest to consider the implementation of NGS in routine prenatal screening including analysis of the COL2A1 gene.

P1136 - Significance of chromosome 18p duplication in prenatal diagnosis

Chariyawan Charalsawadi [1], Chusana Petpichetchian [2], Areerat Hnoonual [1], Pornprot Limprasert [3]
Prince of Songkla University, Pathology, Songkhla-Thailand [1] Prince of Songkla University, Obstetrics and Gynecology, Songkla-Thailand [2] Prince of Songkla Universtiy, Pathology, Songkla-Thailand [3]

Abstract
Duplication of the short (p) arm of chromosome 18 is a rarely reported chromosomal aberration. The disorder is associated with variable phenotypes, ranging from normal to dysmorphisms with mild to moderate intellectual disability. We report on a pregnant woman whom noninvasive prenatal testing (NIPT) indicated chromosome 18p duplication. Ultrasonography revealed isolated bilateral clubfeet. Subsequent Quantitative fluorescent PCR, conventional karyotype, and chromosomal microarray (CMA) analyses on amniotic fluid sample revealed a de novo chromosome 18p duplication. Two duplicated segments were identified, including a segment of 3.043 Mb (18p11.31-p11.23) and a segment of 5.077 Mb (18p11.22-p11.21), encompassing 37 OMIM genes. In addition, we obtained data from 16 previously reported cases that were diagnosed using CMA, include 8 postnatal cases and 8 prenatal cases, to review clinical and genetic findings. Among 17 cases, including our case, the duplicated segments ranged between 428 kb and 14.9 Mb. Approximately 47% of the cases had smaller duplication (< 3.3 Mb) and the remaining cases had larger duplication (>8 Mb). Approximately 47% of the cases, the duplicated chromosome was also detected in a parent. Mild to moderate intellectual disability was noted in 77% of postnatal cases. No fetal abnormalities were detected in 43% of prenatal cases. Among prenatal cases, 4 cases had NIPT result suggested of duplication of chromosome 18p. Six cases decided termination of pregnancy, while 3 cases continued pregnancy and gave birth to a healthy born baby with normal development on a follow-up session. Approximately 76% of the cases had LAMA1 gene duplication but cerebellar dysplasia or hypoplasia was observed in 2 cases with small duplications (15%). We conclude that genomic location and size of the duplicated segment, as well as genes content may not correlate with clinical outcomes.
Keywords: chromosome 18p duplication, trisomy 18p, prenatal diagnosis, chromosomal microarray, non-invasive prenatal

P1143 - Prenatal DiagnosisIAGNOSIS IN A CARRIER OF AN UNSOLVED CRYPTIC TRANSLOCATION

Ester Margarit [1], M. Ángeles Villar [1], Laia Rodríguez-Revenga [1], M. José Rubio [1]
Hospital Clínic, Biochemistry and Molecular Genetics, Barcelona-Spain [1]

Precise characterization of chromosome and regions involved in a determined rearrangement is important for carrier reproductive counselling and prenatal diagnosis. Here we present the case of a pregnant woman, carrier of an undetermined translocation involving the short arm of chromosome 8, who demanded prenatal diagnosis. The objective was the rapid characterization of the maternal cryptic translocation to allow prenatal diagnosis and genetic counselling. for this purpose, we used three complementary cytogenetic techniques: karyotyping, FISH (fluorescent in situ hybridization) and array, to analyze both mother and fetus
Maternal results: karyotype 46,XX,8ps. The satellites present at the short arm of chromosome 8 suggested a cryptic translocation between 8p and the short arm of an acrocentric chromosome. Additionally, one of the chromosomes 21 had no satellites (21ps-), so an 8p;21p translocation was suspected. FISH using a painting probe specific for chromosome 8 allowed the detection of a small hybridization signal on the short arm of the 21ps- chromosome, confirming a maternal cryptic 8p;21p translocation. Array result was normal, indicating that the rearrangement of the mother was balanced, as expected.
Fetal results: karyotype 46,XY,8ps mat. The maternal chromosome 8ps was present in the fetus, but not the 21ps-. FISH analysis confirmed the absence of the rearranged chromosome 21. Array results allowed the definition of fetal chromosome imbalance: del 8p23.3p23.2. This 2,3 MB microdeletion, not detectable at microscopy resolution, includes genes: DLGAP2, CLN8, ARHGEF10 and was considered probably pathogenic, associated to developmental delay, intellectual disability and conduct disorder.
The combination of diverse complementary techniques in this case allowed to resolve the cryptic maternal translocation and the fetal diagnosis, making possible reproductive counselling and prenatal/preimplantational diagnosis for future gestations.

P1145 - A challenge in prenatal diagnosis clinic significance of a CNV in DMD gene (Incidental finding)

Neus Castells-Sarret [1], Sara Perez-Mas [1], Joan Colomer [1], Marina Singla [1], Mar Xunclà [1], Natàlia Rey [1], Eulàlia Rovira [1], Anna Abulí [1], M.Àngels Rigola [2], M. Angeles Sanchez-duran [3], M. Teresa Avilés [3], Alberto Plaja [1], Elena Garcia-Arumí [1], Eduardo Tizzano [1]
Hospital Universitari Vall D'Hebron, Department of Clinical and Molecular Genetics, Barcelona-Spain [1] Universitat Autònoma De Barcelona, Department of Cellular Biology and Medical Genetics, Barcelona-Spain [2] Hospital Universitari Vall D'hebron, Department of Obstetrician, Barcelona-Spain [3]

Introduction: In a prenatal context, the prediction of the clinical significance of a copy number variation (CNV) in the dystrophin gene (DMD) is challenging. CNVs of the DMD gene are the main genetic alteration associated with dystrophinopathies, a wide spectrum of X-linked muscle disease ranging from mild to severe that includes Duchenne (DMD) or Becker (DMB) muscular dystrophy, DMD-associated dilated cardiomyopathy, and subclinical DMB. Correlation between genotype and phenotype is usually established by the reading frame rule (loss of reading frame correlates with severe phenotype); nonetheless exceptions to the "reading frame rule" have been documented, especially with BMD phenotype.
Patients (fetuses and their families) and Methods: We present 4 male fetuses with exonic CNVs involving the DMD gene detected as incidental findings in the course of routine prenatal diagnosis with an arrayCGH of exonic resolution on this gene (60k, CytoSure Constitutional v3 array, ogt).
Results: The prediction method of the reading frame affectation, official DMD databases and family study after the detection in the fetuses of possible carriers has clarified the clinical significance of the different CNVs, thus enabling professionals to establish appropriate genotype-phenotype correlations.
Conclusions: When a CNV in the DMD gene is detected in a male fetus in a prenatal context a fast and systematic multidisciplinary coordination strategy is necessary. The involvement of different medical and laboratory specialists should be considered so as to carry out an exhaustive and simultaneous genetic and clinical study of possible carriers in the maternal side of the family, especially of male relatives, with the purpose of being able to establish a genotype-phenotype correlation for a posterior accurate prenatal genetic counseling.

P1152 - UPD in the Prenatal setting The Benefit of Integration of Methods

Ayala Frumkin [1], Hagit Daum [1], Vardiella Meiner [1], Simcha Yagel [2], Hagar Mor-Shaked [1], Shiri Shkedi Rafid [1]
Hadassah Medical Organization and Faculty of Medicine, Hebrew University of Jerusalem, Genetics, Jerusalem-Israel [1] Hadassah Medical Organization and Faculty of Medicine, Hebrew University of Jerusalem, Obstetrics and Gynecology, Jerusalem-Israel [2]

Introduction
Prenatal detection of uniparental disomy (UPD) is methodologically challenging. Advanced genomic testing in pregnancy may incidentally identify UPD, which poses even greater challenges to both clinicians

and couples, mainly in cases of rare UPDs with scarce published data.
Aim
To demonstrate the benefit of using integration of advanced genomic technologies in an attempt to unravel the clinical significance of a rare UPD in a viable fetus.
Methods:
Amniotic-fluid-DNA was analyzed using Chromosomal microarray analysis(CMA)-GeneChipCytoScan-750K array, followed by Exome analysis using the IDT (xGen Exome Research Panel v2.0 combined with xGen Human mtDNA Research Panel v1.0) capture on an Illumina NOVASEQ 6000.
Results:
A 28-year-old woman at 32 weeks of gestation was referred to late amniocentesis due to severe IUGR in her fetus.
Microarray analysis did not find any pathogenic copy number variants (CNVs), but revealed loss of heterozygosity for extremely large areas on both arms of chromosome 6; altogether about 93.4Mbp of homozygous regions.
A trio Exome sequencing analysis showed a maternal UPD of chromosome 6, with mixed isodisomy and heterodisomy with no overlapping pathogenic CNV or Single nucleotide variants.
Based on no known adverse consequences of maternal UPD of chromosome 6, no bi-allelic pathogenic variants in any of the currently known genes on chromosome 6, and no additional sonographic findings, the couple chose to continue the pregnancy. A cesarean section was performed at 37 weeks and an apparently healthy baby girl was born weighing 1750 grams.
Conclusions
Amniocenteses may pose challenges for genetic counselling, given the complexities and uncertainties unraveled in light of advancing genomic technology. An integrated use of CMA and Exome analysis, together with strict ultrasound surveillance, enables a comprehensive understanding of incidentally identified UPD.
The Exome sequencing gave us some details as to the exact mechanism of the UPD formation. The detailed information enabled the couple to make an informed decision on the future of their pregnancy. Our report adds to the limited data on maternal UPD of chromosome 6 and its mechanism.

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6. Tumour Cytogenomics

P1004 - The importance of "Complex Karyotype" (CK) diagnosis in the Chronic Lymphocytic Leukemia (CLL) patients with normal TP53 FISH (Fluorescence in situ hybridization) results

Yarin Hadid [1], *Mira Ziv [1]*, Adel Shalata [1]
Bnai Zion, Genetic Lab, Haifa-Israel [1]

Introduction: The gold standard cytogenetic method currently used in the diagnosis of CLL patients is FISH for the four specific genetic alterations (TP53, ATM, 13q, Cent12).
Recently, because of the incorporation of "cellular stimulation techniques" into the modern protocols, karyotype testing has become highly relevant for prognosis in CLL patients.
Per definition-CK is diagnosed when three or more chromosomal changes are detected (either structural or quantitative) in a karyotype. CLL patients with CK represent a heterogeneous group with variable clinical presentations.
High-CK (5 or more changes) embody a negative prognostic factor per se. In contrast, intermediate- CK (4 changes) and low-CK (3 changes) represent an aggressive disease only when additional alterations are present (such as mutant IGH status, TP53 deletion or point mutation, etc.).
Methods: We have examined 190 CLL patients in the Genetic Institute in Bnai-Zion Medical Center, from 2016, with the utilization of two methods- FISH and karyotype after B cell stimulation (CpG/IL-2).
Results: Cytogenetic karyotype changes were detected in 111 (58%) out of 190 patients. 27% of the positive tests exhibited CK (high CK- 17%, intermediate- CK 5%, low- CK -5%).
23 tests out of 190 exhibited P53 deletion (12%). Out 167 tests with normal P53, 22% showed CK (14% with high- CK).
Conclusions: Currently there is a scarcity of awareness concerning the importance of karyotyping in the establishment of prognosis and treatment considerations in CLL. The vast majority of hematology laboratories perform FISH testing exclusively.
TP53 deletion is considered an independent negative prognostic factor, similarly to high- CK. In the analysis of the 167 FISH samples negative for TP53 deletion, it was established that in 22% of the cases CK would have been undetected if we had performed FISH testing only. Moreover, 14% of the patients with high- CK would have been undetected, which is a disturbingly high rate.
We conclude that the detection of intermediate and low CK alteration is of critical importance, since these changes tend to develop into high-CK.

P1018 - The role of cytogenetic analysis in patients receiving CAR T cell therapy

Christos Varelas [1], Eleni Gavriilaki [1], Despoina Mallouri [1], Panagiotis Dolgyras [1], Eleni Papchianou [1], Zoi Bousiou [1], Ioannis Batsis [1], Evangelia Yannaki [1], Giorgos Papaioannou [1], Maria Gkaitatzi [1], Michalis Iskas [1], Alkistis Panteliadou [1], Olga Asteriou [1], Anastasia Tsimoglou [1], Ioanna Sakellari [1], *Anastasia Athanasiadou [1]*
Papanicolaou General Hospital, Hematology and Hct Unit, Thessaloniki-Greece [1]

Chimeric antigen receptor (CAR) T-cell therapy can induce durable remissions of relapsed/refractory B-acute lymphoid leukemia (ALL) and B-cell lymphomas. The aim of this study is to examine the value of cytogenetic analysis before CAR T-cell administration, in the context of preexisting chromosomal instability and/or abnormal clones. Our study included 30 patients (female:13, male:17) with a median age of 46 years old (range: 21-75). Four were diagnosed with relapsed/refractory B-ALL and 26 with B-cell lymphomas (DLBCL, MCL, PMBCL). Median number of previous lines of treatment was 4 (range 2–7). Cytogenetic analyses were conducted on bone marrow cells after unstimulated culture (24 & 48hours) and stimulated 72h culture with PHA+IL2. Cytogenetic analysis was available in 22. Twenty one patients had normal karyotype. One patient with Mantle cell lymphoma had complex karyotype with t(11;14) and died after three months due to disease progression. Ten patients with normal karyotype presented with single cell abnormalities (sc ab) 1-6 (median 3 sc ab) in a median follow up of 15,4 months (range 4-23). Six relapsed and 4 died with the probability of overall survival being 49,4% in 2 years. Eleven patients had normal karyotype without sc ab. Five relapsed and 4 died in a median follow up of 3 months (range 1-33) and the probability of overall survival being 52% in 3 years. One of the eight patients without available cytogenetic analysis developed myelodyspalstic syndrome with abnormal clone, after 6 months and is alive at a 22 months follow up. CAR T-cell therapy shows promising results in patients with relapsed/refractory B-ALL and/or B-cell lymphomas. Cytogenetic analysis provides us with valuable information. Larger cohorts with a longer follow-up are needed to determine the contribution of cytogenetic in these patients.

P1019 - Additional cytogenetic abnormalities in patients with core binding factor AML. Experience of a center.

Christos Demosthenous [1], *Giorgos Papaioannou [1]*, Chrysavgi Lalayanni [1], Maria Gkaitatzi [1], Michalis Iskas [1], Christos Varelas [1], Maria Papathanasiou [1], Aliki Tsompanakou [1], Vassiliki Douka [1], Zografia Lazarou [1], Apostolia Papalexandri [1], Tasoula Touloumenidou [1], Ioanna Sakellari [1], Anastasia Athanasiadou
[1]Papanicolaou General Hospital, Hematology and Hct Unit, Thessaloniki-Greece [1]

Core-binding-factor (CBF) AML harboring inv(16) or t(8;21) is classified as favorable risk; nevertheless, relapse rate fluctuates between 30%-40%. Patients with CBF- AML behave differently than other subtypes of AML and need a more sophisticated categorization. In this report, we have retrospectively analyzed the presence of additional cytogenetic abnormalities (ACAs) in patients with CBF-AML. Data on 61 patients diagnosed in our institution between 2000 and 2020 were collected. Bone marrow aspiration samples were assessed by morphology, flow cytometry, cytogenetic and molecular analysis. The median age of the patients was 41 years. Thirty-three had inv(16) and 28 t(8;21). ACAs were identified in 36.3% of patients with inv(16) and 67.8% with t(8;21), p=0.014. Hypodiploidy was identified only in patients harboring t(8;21). Complex karyotype was determined in 2/33 of patients with inv(16), and in 5/28 with t(8;21). The most common ACAs in patients with inv(16) were +8 and +21. Among individuals carrying t(8;21), the most common ACA was -Y followed by del(9q). Overall, loss of sex chromosomes, +4, del(9q), del(7q) were observed only in t(8;21), while +21 and +22 only in inv(16). Clinical information was available for 59 patients with median follow-up of 73.4 months. All achieved CR following intensive chemotherapy. Fourteen patients with inv(16), and 10 with t(8;21) eventually relapsed. Univariate analysis revealed differences in 5-year-OS between patients with inv(16), inv(16) with ACAs, t(8;21), and t(8;21) with ACAs (90%, 72.7%, 44.4%, 72.4%, respectively). Similar differences were also observed in 5-year-DFS. Multivariate analysis did not reveal any statistically significant factor. Overall, CBF-AML with inv(16) or t(8;21) are cytogenetically heterogeneous diseases, whereas 50.8% of patients present with ACAs, more commonly patients with t(8;21). Further research is required to elucidate the biological and prognostic impact of ACAs on the disease outcome.

P1020 - Common ALL and pre B ALL with t(8;14) and t(14;18)

Maria Gkaitatzi [1], Giorgos Papaioannou [1], Christos Varelas [1], Christos Demosthenous [1], Chrysavgi Lalayanni [1], Antonia Syrigou [1], Anastasia Marvaki [1], Chrysanthi Vadikoliou [1], Michalis Iskas [1], Ioanna Sakellari [1], Anastasia Athanasiadou [1]
Papanicolaou General Hospital, Hematology and Hct Unit, Thessaloniki-Greece [1]

De novo MYC and BCL2 rearrangements are rare in pre-B and common acute lymphoblastic leukemia (ALL). This case report presents two patients with these findings and discusses their correlation with clinical outcomes. An 18-year-old male presented with fever, fatigue, blurred vision, and extensive lymphadenopathy. Significant laboratory results were anemia, thrombocytopenia, and high LDH. Bone marrow aspirate revealed 87% lymphoblasts. The immunophenotypic analysis confirmed the diagnosis of pre-B ALL. The karyotype was 46, XY,t(8;14)(q24;q32),t(14;18)(q32;q21)[20]/46,sl,del(6)(q21q23)[3] and FISH analysis revealed MYC and BCL2 rearrangements. The patient was initially treated according to the BFM protocol, but disease progression occurred. Furthermore, the patient showed clinical signs of central nervous system (CNS) involvement. Treatment switched to R-HyperCVAD. After receiving 2 cycles of treatment, he achieved

hematological CR and MRD negativity. Due to the poor prognostic features of the disease, the patient underwent allo-HSCT (sibling) but relapsed after two months. This was followed by chemotherapy, inotuzumab-ozogamicin, and CAR T-cells, but the patient relapsed again after four months and died due to disease progression. A 47-year-old female presented to our department with fatigue, gum hypertrophy, anemia, neutropenia, and high LDH. Bone marrow biopsy revealed 90% lymphoblast infiltration. Flow cytometry confirmed the diagnosis of common ALL. The karyotype was 47,XX,del(3)(q27),+?del(4)(q13q32),t(8;14)(q24;q32),der(13)del(13)(q12q12)del(13)(q22q33),t(14;18)(q32;q21)[cp18]/46,XX[2]. FISH analysis revealed MYC and BCL2 rearrangements. The patient achieved hematological CR and MRD negativity after 2 cycles of treatment (R-CVAD, R-VP/AraC/DXM) and continues their treatment. Common ALL and pre-B ALL with t(8;14)and t(14;18) are rare, and only eight cases have been reported and are associated with refractoriness to standard treatment. These patients may benefit from intensive chemotherapy, followed by allo HSCT. The use of experimental treatment seems to be necessary for this type of leukemia.

P1022 - Optical Genome Mapping in Routine Cytogenetic Diagnosis of Acute Leukemia

Gwendoline Soler [1], Zangbéwendé Guy Ouedraogo [1], Carole Goumy [1], Benjamin Lebecque [2], Gaspar Aspas Requena [3], Aurélie Ravinet [3], Justyna Kanold [4], Lauren Véronèse [1], Andrei Tchirkov [1]
CHU Clermont Ferrand, Cytogénétique Médicale, Clermont Ferrand-France [1] CHU Clermont Ferrand, Hématologie Biologique, Clermont Ferrand-France [2] CHU Clermont Ferrand, Hématologie Clinique Adulte Et De Thérapie Cellulaire, Clermont Ferrand-France [3] CHU Clermont Ferrand, Service D'hématologie Et D'oncologie Pédiatrique Et Unité Creche, Clermont Ferrand-France [4]

Cytogenetic aberrations are found in 65% of adults and in 75% of children with acute leukemia. Specific aberrations are used as markers for the prognostic stratification of patients. The current standard cytogenetic procedure for acute leukemia is karyotyping in combination with FISH and RT-PCR. Optical genome mapping (OGM) is a new technology providing a precise identification of chromosomal abnormalities in a single approach. In our prospective study, the results obtained using OGM and routine techniques were compared in 29 cases of acute myeloid (AML) or lymphoblastic leukemia (ALL). OGM detected 73% (53/73) of abnormalities identified by standard methods. In AML cases, two single clones and three subclones were missed by OGM, but the assignment of patients to cytogenetic risk groups was concordant in all patients. OGM identified additional abnormalities in six cases, including one cryptic structural variant of clinical interest and two subclones. In B-ALL cases, OGM correctly detected all relevant aberrations and revealed additional potentially targetable alterations. In T-ALL cases, OGM characterized a complex karyotype in one case and identified additional abnormalities in two others. Thus, OGM confirmed its performance and its higher resolution allowing the detection of clinically relevant aberrations. In conclusion, OGM is an attractive alternative to current multiple cytogenetic testing that simplifies the workflow for the diagnosis of acute leukemia.

P1023 - Ring chromosomes in hematological malignancies are mainly associated with myeloid malignancies and complex karyotypes

Paraskevi Diamantopoulou[1], Maria Ioanna Margariti [1], Domna Pantelia[1], Marina Kalomoiraki[1], Panayotis Nakopoulos[1], Constantina Sambani[1], *Kalliopi Manola[1]*
Ncsr Demokritos, Cytogenetic, Athens-Greece [1]

Ring chromosomes are uncommon and poorly investigated cytogenetic abnormalities in hematological malignancies.
We investigated the nature of ring chromosomes and their most common additional chromosome abnormalities in patients with hematological malignancies by karyotype and FISH.
The cytogenetic analysis was performed on unstimulated bone marrow cultures using GTG-banding. Metaphase FISH was performed using probes for the centromeres of chromosomes (CEP) 1, 3, 6, 7, 8, 9, 11 and regions 8q24.21/MYC gene, 11q23/MLL gene and 17p13/p53 gene, chosen according to karyotype and diagnosis.
We identified 40 among 28.881 patients (0.14%) with ring chromosomes; 48.5% with acute myeloid leukemia (AML), 33.5% with myelodysplastic syndromes (MDS), 6% with Myeloproliferative Disorders (MPD) and 12% with lymphoid malignancies. The sex ratio was 1.1 (21 males/19 females) and the mean age 67.6 years (12y-91y). Ring chromosomes were found as a sole abnormality in 2 patients (5%), with one more abnormality in 4 (10%) and in complex karyotypes in 34 patients (85%). The most frequent additional abnormalities were marker chromosomes (51.2%), -5/del(5q) (43,8%), -7/del(7q) (34,1%), -11 (26.8%), +8 (24.3%), -21 (19.5%), -17 (17.1%). The nature of ring chromosomes was identified in 16 cases showing: a) CEP 11 accompanied by MLL amplification in 9/16 (56.3%) all in complex karyotypes in AML or MDS, b) MYC amplification without CEP8 in 2/16 (12.5%), c) CEP 7 in 2/16 cases (12.5%), e) CEP 1, 3, 6, 9 and TP53 gene in 1/16 (6.3%) each.
In conclusion, ring chromosomes are very rare aberrations in hematological neoplasms, observed mainly in complex karyotypes and in elderly patients with myeloid malignancies. The most common origin of ring chromosomes was that of chromosome 11, accompanied by MLL amplification. The most

frequent additional abnormalities are marker chromosomes, -5/del(5q) and -7/del(7q).

P1024 - Optical genome mapping in Leukemia demonstrates full concordance and new cytogenetic findings in an Israeli cohort

Nivin Moustafa-Hawash [1], Christina Srouji [1], Avraham Frisch [2], Nira Arad-cohen [3], Karin Weiss [1], Tamar Paperna [C]
Rambam Health Care Campus, Genetic Institute, Haifa-Israel [1] Rambam Health Care Campus, 2Department of Hematology and Bone Marrow Transplantation, Haifa-Israel [2] Rambam Health Care Campus, Pediatric Hematology-oncology Department, Haifa-Israel [3]

Background:
Acute lymphoblastic and myeloid leukemia (ALL, AML) are characterized by a large number of genetic aberrations which can provide prognostic information. Current guidelines indicate the use of several cytogenetic methods to identify structural variants (SV). Optical genome mapping (OGM) is a promising alternative which uses long labeled single DNA molecules to identify SVs at an unprecedented resolution.
Objective
To evaluate the utility of OGM in the cytogenetic workup of AML/ALL, in comparison to state-of-art cytogenetic tests in a clinical laboratory.
Methods
Bone marrow aspirates were obtained from 31 patients with leukemia (11 pediatric B/T-ALL, 20 adult AML/ALL) at diagnosis. Samples underwent routine cytogenetics testing (Karyotype, FISH and/or CMA). for OGM, DNA was extracted, labeled, imaged, and analyzed using recommended procedures. Rare variant and De Novo analyses were employed. Aneuploidies, translocations, large and smaller SVs were recorded, and then compared to the original cytogenetics results.
Results
OGM analyses detected all previously reported cytogenetic abnormalities from the routine tests. The variant allele frequency detection limit by OGM was ~5-10%, depending on SV size. Turnaround time from sample to result was 7-15 days.
In 16/31 cases analyzed, OGM detected new translocation fusion partners, solved the origin of marker chromosomes previously identified in karyotype, and identified new gene deletions. Importantly, these included malignancy-associated SVs that clarified prognosis and response to therapy. for example, deletion of IKZF1 gene in ALL changed prognosis to high risk and accordingly- treatment.
Conclusions
OGM demonstrated full concordance with routine cytogenetic testing. The rate of new findings highlights the potential of OGM to identify novel SVs for evaluation as new prognostic and therapeutic markers.
We suggest OGM can reliably replace the cytogenetics methods used currently, as a first line test in hematology diagnostics.

P1027 - The importance of using FISH (Fluorescent In Situ Hybridization) technology as an investigative tool in patients with malignant hemopathies in Albania

Dorina Roko [1], Anila Laku-Babameto [1]
University Hospital Center “Mother Teresa”, Genetic Laboratory Service, Tirana-Albania [1]

Fluorescent in Situ Hybridization (FISH) analysis offers one of the most sensitive, specific, and reliable strategies for identifying acquired genetic abnormalities, such as characteristic gene fusions and loss of a chromosomal region associated with hematologic disorders.
This study aimed to use different FISH probes, such as BCR/ABL, AML1/ETO, PML/RARA, IGH/MYC, P53, etc., as a diagnostic tool and suggest prognostic outcomes in Chronic Myeloid Leukemia (CML), Acute Myeloid Leukemia (AML), Chronic Lymphoblastic Leukemia (CLL), Acute Lymphoblastic Leukemia (ALL), Myelodysplasia (MDS), Multiple myeloma and Lymphoma cases.
We report the results of interphase FISH (iFISH) analysis of 115 patients with a known hematological disorder, including 56(48.6%) CML, 11(9,5%) AML, 17(14,7%) CLL, 5(4,3%) ALL, 16(13,9%) MDS, 7(6%) Multiple Myeloma and 3(2,6%) Lymphoma patients diagnosed clinically by the Haematological Service in the University Hospital Center “Mother Teresa” Tirana. Then FISH analysis was performed in the Molecular Cytogenetic Laboratory.
In our study, FISH analysis from Cytocell Company provides a rapid and reliable method for the determination of specific translocations such as t (9;22) in 17(30,3%) of CML patients, t (8;14) in one (5,8%) of CLL patient, t (15;17) in one (9%) of AML and in one (20%) of ALL patients that have been shown considerable prognostic significance and are used as measurable targets for therapy responses.
We detected 17p deletion in 3(17,6%) of CLL patients and 2(12,5%) of MDS patients, suggesting a pathogenetic role for inactivation of tumor suppressor genes located in 17p and have been associated with poor prognoses.
In addition, one patient with AML showed trisomy 8, trisomy 21, and tetrasomy 21 in conventional cytogenetic and FISH analysis, which has always been regarded as a poor prognostic indicator to show very low complete remission rate and overall survival.
In conclusion, our results confirmed that iFISH had become an invaluable tool in defining and monitoring structural chromosome abnormalities and is a sensitive technique to detect numerical aberrations for monito-

ring response to therapy as well as minimal residual disease.

P1033 - Cytogenetic profile of Core Binding Factor Acute Myeloid Leukemia in Tunisian patients

Wiem Ayed [1], Meriem Youssef [1], Rachid Kharrat [2], Afef Jelloul [1], Helmi Guermani [1], Nabila Abidli [1], Imen Charfi [1], Manel Gharbi [1], Rayhan Belakhal [3], Ahlem Amouri [1]
Institut Pasteur De Tunis, Histology and Cytogenetics, Tunis-Tunisia [1] Aziza Othmana Hospital, Clinical Hematology,, Tunis-Tunisia [2] Aziza Othmana Hospital, Clinicial Hematology, Tunis-Tunisia [3]

The core binding factor (CBF) is a complex protein that plays an important role in myeloid differentiation. It is recurrently altered in acute myeloid leukemia (AML) through genomic rearrangements: RUNX1/ RUNX1T1 fusion from t(8;21)(q22;q22)and CBFB/ MYH11 from inv(16)(p13.1q22)/t(16;16)(p13.1;q22). The resulting CBF-AML constitutes 10–15% of adult de novo AML cases and is associated with a favorable prognosis.
Here, we carried out a retrospective study of 79 patients with CBF-AML referred to our laboratory for cytogenetic study between January 2012 and December 2021.
After short-term culture, a conventional karyotype was performed on the BM aspirate and analyzed after R-banding.
CBF-AML was reported in 25,48% of all AML cases. The median age was 32.3 years. The sex ratio was 1,02.
The t(8;21)(q22;q22) were observed in 44 patients. In more than half of cases (59.09%), karyotype showed the presence of additional cytogenetic anomalies (ACA). The main ones were the loss of Y and X gonosomes, followed by del(9q)(q21q22)).
the inv(16)(p13;q22) or t(16;16)(p13;q22) were found in 35 paients. for some patients, FISH experiments were carried out and demonstrated the presence of CBFB-MYH11 fusion gene. ACA were observed in 57.14% of patients. Main anomalies were trisomy 8, trisomy 22 as well as del(7q)(q21q31).
The evolution was favourable in the majority of cases (75%), showing complete cytogenetic remission. Relapses were reported in 13 patients (16.45%).
Our results confirmed the favorable prognosis of CBF-AML despite the presence of ACA and the role of cytogenetic analysis for the diagnosis and prognosis of the CBF AML.

P1039 - Presence of JAK2 V617F MPL mutations and cytogenetic aberrations in patients with thrombocytosis

Milica Strnad [1], Biljana Todoric-zivanovic [1], Dragana Stamatovic [2], Marija Elez [2]
Mma, Institute of Pathology, Beograd-Serbia [1] Mma, Clinic of Hematology, Beograd-Serbia [2]

Background: Thrombocytosis is seen as a primary disease - myeloproliferative neoplasm (MPN), or a reactive (secondary) disease of the bone marrow. Approximately half of all ET patients have a mutation of the JAK2V617F gene, 3-8% MPL mutations, and 25-35% mutations in the calreticulin (CALR) gene.
Aim: We aimed to determine the presence of JAK2V617F mutation in all patients with thrombocytosis and to analyze the presence of MPL mutations in some patients without JAK2V617F mutation.
Material and Methods: In this study, patients who were sent for examination to our laboratory with thrombocytosis (platelet count ≥450 x 109/L) between 2012 and 2022 were analyzed. Cytogenetic studies were carried out on Gimsa-banded chromosomes obtained directly and from 24-hour unstimulated bone marrow cultures. The mutation of JAK2V617F was determined by ARMS-PCR analysis. Mutations of MPL (W515L and W515K) were determined by competitive allele-specific RQ-PCR analysis (mutation assay).
Results: We retrospectively evaluated 606 patients with thrombocytosis. JAK2V617F mutation was positiv in 315 (52%) of cases. MPL mutations were analyzed in 30 patients, and no mutations were detected. Cytogenetic aberrations were not detected in any patients.
Conclusions: Analysis of JAK2V617F mutation is a necessary procedure to diagnose primary thrombocytosis in patients with persistent thrombocytosis after excluding the causes of secondary thrombocytosis. The incidences of MPL mutations are too low to become routine clinical practice. A positive result for either JAK2 or MPL mutation confirms the presence of a myeloproliferative neoplasm. Still, we need a combination of clinical, morphological, and molecular genetic features to distinguish one type from another.

P1045 - Chromosome 1 abnormalities in Childhood B Lymphoblastic Leukemia – An analysis with respect to clinical variables and survival outcome

Neelum Mansoor [1], Sidra Maqsood [2], Syeda Ambareen Zehra [3], Naila Rafiq [4], Azfar Ahmed [3], Aamir Ehsan [5]
Indus Hospital and Health Network, Hematology and Cytogenetics, Karachi-Pakistan [1] Indus Hospital and Health Network, Research, Karachi-Pakistan [2] Indus Hospital and Health Network, Cytogenetics, Karachi-Pakistan [3] Indus Hospital and Health Network, Pediatric Oncology, Karachi-Pakistan [4] Corepath Laboratories, Cytogenetics, San Antonio-United States [5]

Background: Chromosome 1 abnormalities (C1As) are common genetic aberrations in hematological malignancies. We sought to evaluate the significance of these abnormalities with reference to clinical

characteristics and survival outcome in a pediatric B-Lymphoblastic Leukemia (B-ALL) cohort.
Methods: This is a retrospective, observational study conducted in hematology department of Indus Hospital and Health Network. Following ethical approval, the hospital's electronic medical record was reviewed from October 2020 to July 2022. Data extracted for childhood B-ALL cases exhibiting C1As. Chromosome analysis performed on Cytovision MB8 using G-banded metaphases derived from unstimulated bone marrow culture. Results were recorded according to the International System for Human Cytogenetic Nomenclature (ISCN-2020). Data analyzed using SPSS, version 24.0.
Results: C1As were observed in 60/450 (13.3%) cases of B-ALL. Among C1As, 29 (48%) cases had t(1;19). There were 13 (45%) balanced and 16 (55%) unbalanced translocations. The aberrations without t(1;19) were seen in 31 (52%) cases including 1q duplication with concomitant hyperdiploidy and complex karyotype in 14 (45%) and 4 (13%) cases respectively. Deletion 1p and three-way translocations observed in <5% cases. The common break points were q21 in 30(49.2%) and q23 in 15(24.6%) cases. The median age for C1As with and without t(1;19) was 8 years versus 6 years (p=0.018) while the median leukocyte count was 32 x 109/L versus 17 x 10E9/L (p=0.042). Disease-free survival (DFS) for cases with t(1;19) was 72% (62% versus 81% in balanced versus unbalanced translocations) while it was 74% for cases without t(1;19). Median follow-up was 14 months.
Conclusion: C1As are widely studied in multiple myeloma and solid tumors however, data is limited in childhood B-ALL. Our study demonstrates that this genetic aberration often occurs within this entity. The high frequency of 1q duplication and the survival difference in the balanced and unbalanced t (1;19) are the study highlights, hence justify large-scale studies for further insight.

P1051 - Additional chromosomal abnormalites in Philadelphia chromosome positive chronic myeloid leukemia. Single center experience.

Arpad Szomor [1], Zsuzsanna Szijarto [2], Agnes Nagy [1], Szabolcs Kosztolanyi [1], Judit Pammer [1], Bela Kajtar [3], Hussain Alizadeh [1]
University of Pecs, First Department of Medicine, Pecs-Hungary [1] United Health Institute, Ophthalmology, Pecs-Hungary [2] University of Pecs, Department of Pathology, Pecs-Hungary [3]

Chronic myeloid leukemia (CML)is a myeloproliferative neoplasm which is characterized by a balanced genetic translocation t(9;22) (q34;q11.2). This rearrangement is known as the Philadelphia chromosome. The aim of our study was to analyze the impact of baseline additional chromosomal abnormalities (ACAs) on the choice of the first-line treatment and the outcome in the newly diagnosed patients. We report our findings in 89 patients diagnosed with CML in our center. Eigthy three patients (93,3 %) had t(9;22) (q34;q11) translocation without ACAs (Group A) and 6 patients (6,7 %) had ACAs(Group B). The demographics of patients in Group A was 52,0 years(range 7-80), 44 (53 %) female, 39 (47 %) male. The median age was 48,6 years (range 37-64) in Group B (6 male patients), respectively. Bone marrow karyotyping was performed at diagnosis following 24-48 h culturing and using G-banding. At least 20 methaphases were investigated, clonal aberrations were described following ISCN 2020. Among additional chromosomal abnormality, 3 patients had single: del(20q), t(14;22), t(4;7) - and 3 had complex cytogenetic abnormality. We found transitory additional abnormalities in further 6 patients during therapy. The observation period was 124,7 (range 6-344) months in Group A, 133,6 (range 22-271) months in Group B, respectively. The first-line treatment was tyrosine kinase inhibitor in 78 (87,6 %) patients, and the CML diagnosis in remaining cases was made prior to the introduction of imatinib. Currently, 12 patients are in treatment-free remission. In conclusion, we did not find any prognostic signicance of ACA in newly diagnosed CML.

P1052 - Validation of the OGM for cytogenomic testing in hemato oncology – Sheba Medical Center experience.

Victoria Marcu [1], Mai Milhem [1], Dan Dominissini [2]
Sheba Medical Center, Hematology Laboratory, Tel Hashomer-Israel [1] Sheba Medical Center, Hematology Laboratory, Cancer Research Center, Wohl Institute for Translational Medicine, Tel Hashomer-Israel [2]

Cytogenetic/cytogenomic analysis is highly important for diagnostic, prognostic and therapeutic decisions for hemato-oncologic patients. Chromosomal aberrations in hemato-oncological samples are usually detected using three different tests: karyotype analysis, FISH and Cytoscan array. Optical genome mapping method (OGM) may consolidate and replace these three methods in one test and to provide relatively fast results. Bionano optical genome mapping is a new cytogenomic technology that can detect chromosomal structural and copy number variants at high sensitivities in an unbiased genome-wide manner. The technology creates a consensus genome map from the images of the enzymatically labeled high molecular weight DNA that was linearized into nanochannels by electrophoresis. DNA for the OGM tests can be isolated directly from various biological samples, such as bone marrow aspirate, peripheral blood, biopsies or any enriched cell population (like CD138 positive plasma cells).
Our laboratory has evaluated OGM on 40 hematological samples by testing accuracy and specificity of the method as compared to conventional tests. Mainly, samples of acute leukemia were analyzed. Chromosomal aberrations found by OGM were concordant in 95% of cases, including 100% verification of

aneuploidies. Additional aberrations were detected in 52% of samples, mostly changing the prognosis and the recommended treatment. Importantly, normal karyotype as was diagnosed by conventional G-banding was confirmed by OGM as well. Additional parameters, such as limit of detection and precision of OGM, met our acceptance criteria during evaluation process.
To conclude, OGM indeed can provide unbiased genome-wide detection of chromosomal aberrations, some of which would be missed otherwise using standard methods. Profound advantage of OGM is reducing multiple tests into one. The superior ability to detect structural changes (translocation, inversions, deletions, duplications, etc.) removes the need for reflex FISH tests, reducing the costs, turnaround time and laboratory work load. We plan to expand OGM testing for all hematologic indications and believe it will soon become major testing method along with NGS to define cytogenomic and genetic diagnosis for hematological malignancies.

P1059 - T cell receptor (TCR) loci rearrangements in children with T cell acute lymphoblastic leukemia

Libuse Lizcova [1], Eva Prihodova [1], Lenka Pavlistova [1], Karla Svobodova [1], Lucie Hodanova [1], Ester Mejstrikova [2], Ondrej Hrusak [2], Iveta Janotova [3], Lucie Sramkova [3], Jan Stary [3], Zuzana Zemanova [1]
General University Hospital and First Faculty of Medicine, Charles University In Prague, Center of Oncocytogenomics,institute of Medical Biochemistry and Laboratory Diagnostics, Prague-Czechia [1] Second Faculty of Medicine, Charles University, Prague and University Hospital Motol, Clip - Childhood Leukaemia Investigation Prague, Department of Paediatric Haematology and Oncology, Prague-Czechia [2] Second Faculty of Medicine, Charles Univesity, Prague and University Hospital Motol, Department of Pediatric Hematology and Oncology, Prague-Czechia [3]

T-cell acute lymphoblastic leukemia (T-ALL) is a clinically and genetically heterogeneous disease that constitutes 10%–15% of all newly diagnosed pediatric ALL cases and is caused by the accumulation of genetic abnormalities that alter mechanisms controlling normal T-cell development. The most common structural aberrations involve rearrangements of T-cell receptor (TCR) loci – TRA/TRD (14q11) and TRB (7q34), which are found in ~25% of patients.
The aim of our study was to analyze TCR rearrangements and associated cytogenomic aberrations in children with T-ALL and to correlate the findings with clinical data.
The bone marrow samples of 66 children diagnosed between 1996-2017 (46 boys, 20 girls, median 7.9 years) were analyzed using combination of cytogenomic methods: conventional cytogenetic analyses, I-FISH (Abbott Molecular, Dako), mFISH/ mBAND (MetaSystems), MLPA (MRC Holland) or CGH/SNP array (Agilent).
Rearrangements of TCR loci were detected in 18/66 (27%) patients – TRA/TRD (9x), TRB (5x) and TRA/TRD together with TRB (2x). In 10/18 cases, recurrent affected oncogenes TAL1 (3x), LMO2 (3x), TLX1 (2x), TAL2 (1x) and MYC (1x) were identified. In the remaining cases, TCR rearrangements were cryptic or were a part of complex karyotype. Additional aberrations were detected in all but one patient with the deletion of CDKN2A gene being the most frequent one. Children with aberrations of TCR loci had significantly better prognosis - EFS ($p=0.011$) and OS ($p=0.0074$).
Aberrations of T-cell receptors form a genetically heterogeneous group of rearrangements, leading to aberrant expression of oncogenes involved in T-cell maturation/proliferation. They mostly occur with abnormalities of genes involved in the regulation of the cell cycle and/or signaling pathways, confirming the multistep process of T-ALL pathogenesis. In our cohort, patients with TCR rearrangements had an excellent prognosis regardless of the presence of other aberrations.
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P1060 - Optical genome mapping of bone-marrow in Hematological malignancies

Hila Nachmias [1], Bat Hen Trunoch [1], Hila Kolog [1], Dr. Anat Bar Shira [1], Prof Hagit Baris Feldman [1]
Tel Aviv Sourasky Medical Center Ichilov, Genetic Institute, Tel Aviv-Israel [1]

Introduction: Extreme genomic rearrangements are hallmarks of cancer. AML is characterized by genetic aberrations (chromosomal translocations, deletions, insertions) affecting its classification for risk of treatment. Optical Genome Mapping (OGM) detects structural variations in an unbiased manner and at much higher sensitivities than cytogenetic techniques used to identify those aberrations, such as karyotyping or fluorescence in situ hybridization (FISH).
Materials & Methods: Heparinized bone marrow aspirates were collected from patients with suspected acute myeloid leukemia. (AML) for OGM protocol UHMW gDNA extraction from frozen BMA was performed according to guidelines, and with reagents provided by the manufacturer (Bionano Prep SP BMA DNA Isolation Protocol gDNA was quantified using the Qubit™ dsDNA BR Assay Kit. Fluorescent dye labeling of the UHMW gDNA (specific labeling and whole DNA staining) was performed according to the manufacturer’s guidelines (Bionano Prep Direct Label and Stain (DLS) Protocol. The stained and labeled UHMW gDNA was loaded on a Saphyr G2.3 chip. The Saphyr device imaged the DNA molecules imaged with a maximum capacity of 1500 Gbp per sample. Structural variants were analyzed using the Bionano Access software.

Results: The OGM results were compared to the known aberrations as detected by karyotyping and FISH. The data is summarized.
Discussion: It can be concluded that the results seen by the karyotype and the FISH were all observed using the OGM method; in addition, chromosomal aberrations that could not be diagnosed with low resolution were observed using the OGM. It is necessary to continue performing karyotyping at the same time as the OGM test in order to gain more experience and knowledge.

P1061 - Newly detected TP53 gene deletion in repeatedly examined patients with multiple myeloma (MM)

Lenka Pavlistova [1], Adela Berkova [1], Karla Svobodova [1], Jan Straub [2], Ivan Spicka [2], Libuse Lizcova [1], Zuzana Zemanova [1]
Center of Oncocytogenomics, Institute of Medical Biochemistry and Laboratory Diagnostics, General University Hospital and First Facult Yof Medicine, Charles University In Prague, Prague-Czechia [1] First Department of Internal Medicine-department of Hematology, General University Hospital and First Faculty of Medicine, Charles University In Prague, Prague-Czechia [2]

Deletion of TP53 gene (17p13) is considered high risk chromosomal aberration in patients with multiple myeloma (MM). Diagnostic examination of del(TP53) is an integral part of risk stratification of MM patients. However, the clinical significance of TP53 deletion acquired later in the course of the disease has not yet been studied in detail
During 2005-2022, diagnostic bone marrow samples of 1719 MM patients were examined using FISH on immunofluorescently labeled plasma cells (cIg-FISH) with DNA probes for detection of IgH gene (14q32) rearrangements, monosomy 13/del(13q), gain/deletion of 1q21/1p32 and deletion of TP53 gene.
Altogether, 250/1719 patients were examined repeatedly in relapse or disease progression. TP53 deletion was newly detected in 19/250 cases (7.6%). Gain of other aberrations in addition to del(TP53) was proved in nine cases. Patients were treated with one to four lines of chemotherapy (median 3). The median time to detection of TP53 deletion was 45 months (range 7-180 months). Survival data are available for 18 cases. Five patients are currently alive, thirteen patients died. The median survival since detection of TP53 deletion was 5 months (range 1-56 months), and the median overall survival was 59 months (range 11-116 months).
We detected a new clone with TP53 deletion in 7.6% of repeatedly examined MM patients. In these cases, we confirmed very short survival from the acquisition of TP53 deletion and worse overall survival. In MM, chemotherapy is considered the most important factor selecting hidden subclones with chromosomal aberrations or inducing the development of additional chromosomal changes. Whether our patients underwent classic clonal evolution or expansion of hidden subclones due to chemotherapy will be the subject of further studies.
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P1067 - Donor cell acute myeloid leukaemia after haematopoietic stem cell transplantation for chronic granulomatous disease

Roberto Valli [1], Annalisa Frattini [1], Anna Leszl [2], Marco Zecca [3], Pamela Roccia [4], Paolo Bernasconi [5], Irene Dambruoso [5], Antonella Minelli [6], Giuseppe Montalbano [7], Giovanni Micheloni [7], Giovanni Porta [7], *Francesco Pasquali [7]*
Università Dell'insubria, Genetica Umana E Medica, Dipartimento Di Medicina E Chirurgia, Varese-Italy [1] Università Di Padova, Clinica Oncoematologica, Dipartimento Di Salute Della Donna E Del Bambino, Padova-Italy [2] Fondazione Irccs Policlinico S. Matteo, Pediatric Hematology / Oncology, Pavia-Italy [3] Università Di Varese, Genetica Umana E Medica, Dipartimento Di Medicina E Chirurgia, Varese-Italy [4] Università Di Pavia, Ematologia, Fondazione Irccs Policlinico S. Matteo and Università Di Pavia, Pavia-Italy [5] Università Di Pavia, Genetica Medica, Dipartimento Di Medicina Molecolare, Pavia-Italy [6] Università Dell'insubri, Genetica Umana E Medica, Dipartimento Di Medicina E Chirurgia, Varese-Italy [7]

The patient reported here underwent Haematopoietic Stem Cell Transplantation (HSCT) due to Chronic Granulomatous Disease (CGD), caused by biallelic mutations of the NCF1 gene. Two years later he developed Acute Myelogenous Leukemia (AML) which was unexpected and was recognized to derive from donor cells thanks to sex mismatched chromosomes, being the patient male and the donor his sister. Donor Cell Leukaemia (DCL) is very rare, and it was never reported in patients with CGD after HSCT. In the subsequent ten years AML relapsed three times and the patient underwent chemotherapy and three further HSCT, being donors respectively the same sister of the first HSCT, an unrelated donor, and his mother. The patient died at the third relapse. The DCL was characterized since the onset by an acquired balanced translocation between chromosomes 9 and 11 in bone marrow (BM), with a molecular rearrangement between MLL (alias KMT2A) and MLLT3 genes, quite frequent as cause of AML. All the relapses showed the malignant clone with XX sex chromosomes and this rearrangement, thus indicating that it was always the original one, deriving from the transplanted sister's cells. It showed to be able to remain quiescent in BM during repeated chemotherapy courses, remission periods and HSCT. The leukaemic clone then acquired different additional anomalies, during the ten years of follow-up, with cytogenetic results characterized both by anomalies frequent in AML, as a dicentric isochromosome of the

long arm of chromosome 17, and with different changes not recurrent: two different translocations involving chromosome 2, one balanced, one unbalanced, and a deletion of the short arm of chromosome 12. Such a cytogenetic course is not so common in AML.

P1069 - mutation of the PIK3CA gene in breast cancer.

Gulsim Smagulova [1], Ainur Tulepbayeva [1], Nurlan Nurgaliyev [1], Symbat Kaus [1], Suriya Yessentayeva [2]
Diagnostic Laboratory Gammalab Llp, Molecular Genetic Research, Almaty-Kazakhstan [1] Krmu, Oncology and Mammology With A Course of Visual Diagnostics, Almaty-Kazakhstan [2]

Introduction: According to the statistics of the oncology service of Kazakhstan for 2021, breast cancer in terms of incidence and early detection ranks first among oncology diseases in women. of these, more than 70% are women under the age of 60. and more than 75% of them are patients with luminal A and B type . Activating mutations in the PIK3CA oncogene are often found in hormone-dependent, HER2-negative types of breast cancer. Targeted therapy with a selective inhibitor of the PIK3 alpha isoform is recommended for such patients.
Material and methods: In total, 37 samples of breast cancer were studied, which were conditionally divided into groups: the first group - 12 patients with luminal type A and B after therapy; the second group - 25 patients before any therapy. A commercial allele-specific real-time PCR kit was used to test PIK3CA.
Results: Out of 37 examined samples, 11 had mutations in the PIK3CA oncogene (30%). 8 of which (73%) were due to E542K and E545K mutations. In the first group, out of 12 patients mutation was found in 5 (42%) , in the second, out of 25 patients mutation was found in 6(24%).
Conclusions: Activating mutations in the PIK3CA gene were found in 30% of cases in hormone-dependent and HER2-negative breast cancer.
In the group in which genetic testing was performed after hormone therapy, the percentage of detection of mutated PK3CA was higher than in the group in which PIK3CA testing was performed immediately in conjunction with IHC testing before hormone therapy. Since targeted therapy with a selective inhibitor of the alpha isoforms of PIK3 is recommended for patients with mutated PIK3CA gene, it is advisable to include this genetic testing in the domestic Protocol for the diagnosis and treatment of breast cancer to determine the status of the PIK3CA gene simultaneously with the detection of the tumor immunophenotype.
Tumor cells with mutated PIK3CA oncogene can serve as a good marker for monitoring the effectiveness of targeted therapy by Liquid biopsy (ctcDNA).

P1075 - Optical Genome Mapping use in the multiple myeloma diagnosis

Céline Lété [1], Nicolas Catarin [1], William Llorente [1], Alexandra Guadagni [1], Vincent Bours [1], Mauricette Jamar [1], Pablo Beckers [1], Catherine Menten [1]
CHU Liège, Cytogénétique, Liège-Belgium [1]

Diagnosis and treatment of patient with multiple myeloma (MM) is based on the detection of hyperdiploidy, structural variations (SV) such as IGH rearrangements or copy number variations (CNVs) such as del(17/17p) and gain(1q). Molecular karyotype (array-CGH) and several specific FISH analyses are performed on selected CD138+ cells. However, FISH analyses target only specific gene or rearrangement and array-CGH cannot detect balanced SVs. Moreover, observation and interpretation of FISH profiles are not always easy. Optical genome mapping (OGM), a new cytogenetic technology, allows the detection of (un)balanced structural variations (SV), large CNVs and specific gene gain or loss in a single test. In our study, we used the OGM (Bionano - Saphyr) to determine whether this new technology is an suitable alternative to cytogenetic assessment in MM. Firstly, we analyzed 13 bone marrow samples, with a plasma cell infiltration between 10 to 92%, without prior tumor plasma cell selection. Compared to our standard testing pathway, hyperdiploidy is completely identified in only one sample, partially in 3 samples and not found in 3 samples. Recurrent SVs were correctly observed in 2 samples but the detection of t(11;14) translocation failed in 2 samples. Then, we tested OGM technique on 4 samples with hyperdiploidy after plasma cell enrichment. Different fractions of selected tumor cells were used to test the detection limit of hyperdoiploidy. In fractions between 100% and 50% of plasma tumor cells, hyperdiploidy was detected, but not at 20%. SVs were identified in both the 100% and 20% fractions. In conclusion, the OGM technique could be used in MM diagnosis but requires plasma tumor cell enrichment techniques.

P1077 - NPM1(+) Acute Myeloid Leukemia with an abnormal karyotype

Chrysavgi Lalayanni [1], Anastasia Athanasiadou [1], Michael Iskas [1], Apostolia Papalexandri [1], George Papaioannou [1], Stephania Bountoura [1], Stamatia Sourri [1], Vassiliki Kanava [1], Maria Gkaitatzi [1], Ioannis Batsis [1], Despoina Mallouri [1], Theodolinda Testa [1], Natassa Konstantinou [1], Maria Papathanasiou [1], Anastasia Marvaki [1], Ioanna Sakellari [1]
G Papanikolaou Hospital, Hematology Dpt, Thessaloniki-Greece [1]

Acute Myeloid Leukemia (AML) with mutated NPM1 is a distinct entity, usually presenting a normal karyotype. According to the revised ELN2022 classification, in the absence of FLT3-ITD and adverse-risk cytogenetic abnormalities, NPM1-

mutated AML confers a favorable prognosis. It is unclear if other intermediate-risk abnormalities have a prognostic impact and there are controversial reports in the literature.
We studied 92 patients with NPM1(+) AML (42 men/50 women, median age 57(23-72) years), treated with intensive chemotherapy in our Center. 78 patients had normal and 14 (18%) abnormal karyotype; 27/92 carried the mutation FLT3-ITD (29%). Among the 14 patients with abnormal karyotype, 2 presented high-risk (-7, t(3q26.2;v) and 12 patients intermediate-risk abnormalities, with most frequent the trisomy 8 (6 patients) and del9q (2 patients). Two patients carried a hyperploid karyotype (49-50 and 57 chromosomes). No significant differences were observed in the characteristics of patients with normal or abnormal karyotype such as WBC count, gender and age, nor the FLT3-ITD mutation (32% vs 14%, p=0.3). Among the 65 patients without FLT3-ITD, achievement of complete remission (CR) was significantly higher within the group of 53 patients with a normal karyotype compared to the group of 12 patients with abnormal, intermediate-risk karyotype (98% vs 83%, p=0.019) while the relapse rates did not differ significantly. The 5-year survival rates (OS) were 58% and 41.6% (p=0.4) and the 5-year DFS were 48% and 34.3% respectively (p=0.58).
In conclusion, aberrant karyotype is uncommon in AML NPM1(+), so the number of studied patients was small. Nevertheless, a lower CR rate was observed in NPM1(+) AML with coexisting intermediate-risk karyotypic abnormalities compared to normal karyotype. No significant survival differences could be detected. Larger patient number studies should be conducted to clarify the prognostic value of an abnormal karyotype in NPM1-mutated AML.

P1081 - Hematological disorders in patients with constitutional chromosomal abnormality

Marie Valerianova [1], Iveta Mendlikova [1], Kristina Rochlova [1], Zuzana Zemanova [2], Lenka Pavlistova [2], Jaroslav Cermak [3], Anna Jonasova [4], Sarka Ransdorfova [1]
Ihbt, Cytogenetics, Prague-Czechia [1] General University Hospital and First Faculty of Medicine, Center of Oncocytogenomics, Prague-Czechia [2] Ihbt, Medical Department, Prague-Czechia [3] General University Hospital and First Faculty of Medicine, Medical Department, Prague-Czechia [4]

Introduction: The impact of constitutional chromosomal abnormalities on the pathogenesis of hematological diseases is not yet sufficiently understood. So far, the association with acute leukemia has been clearly demonstrated in trisomy 21 and the rare Robertsonian translocation der(15;21) (q10;q10). The type of disease, clinical history, and acquired chromosomal abnormalities were determined in carriers of constitutional chromosomal aberrations investigated for hematological indications in 1998-2022. This retrospective study aimed to assess hematological malignancies and/or other hematological disorders in relation to congenital karyotype abnormalities.
Materials and Methods: Bone marrow and/or peripheral blood samples cultured for 24 or 48 hours without mitogen were analyzed by cytogenomic methods (G-banding, FISH analyses). Aberrations of potentially constitutional origin were verified on PHA-stimulated peripheral blood lymphocytes.
Results: Constitutional chromosomal aberration concomitant with hematological diseases were randomly identified in 89 patients (40 sex chromosome abnormalities and 49 autosomal aberrations). Cytogenetic examination was indicated in these patients because of various hematological disorders: 34 non-malignant hematological diseases (38%), 37 myeloproliferative (42%), and 18 lymphoproliferative (20%) neoplasms. A combination of congenital and acquired chromosomal aberrations was found in the bone marrow karyotype in 15 patients (17%), most frequently in patients with a reciprocal translocation (8 cases). Five patients with a unique reciprocal translocation were diagnosed with AML with recurrent genetic abnormalities, and three cases had t(8;21)(q22;q22.1); RUNX1::RUNX1T1 fusion gene.
Conclusions: A higher incidence of specific disease in the group of patients with the same type of constitutional change was not observed. A comparison of our and published data shows that the association of hematological malignancy with congenital chromosomal aberration appears to be coincidental. Congenital aberrations are unlikely to be a risk factor for the development of hematological disease, with the exception of acute leukemia and trisomy 21 and the Robertsonian translocation der(15;21)(q10;q10).
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P1082 - Significance of chromosome 7 aberrations in myeloid malignancies

Sarka Ransdorfova [1], Martina Onderkova [1], Marie Valerianova [1], Kristina Rochlova [1], Iveta Mendlikova [1], Libuse Lizcova [2], Lenka Pavlistova [2], Karla Svobodova [2], Lucie Hodanova [2], Jaroslav Cermak [3], Jan Valka [3], Anna Jonasova [4], Zuzana Zemanova [2]
Institute of Hematology and Blood Transfusion, Department of Cytogenetics, Prague-Czechia [1] Institute of Medical Biochemistry and Laboratory Diagnostics, General University Hospital and First Faculty of Medicine, Charles University, Center of Oncocytogenomics, Prague-Czechia [2] Institute of Hematology and Blood Transfusion, Medical Department, Prague-Czechia [3] General University Hospital and First Faculty of Medicine, Charles University, 1st Medical Department, Prague-Czechia [4]

Introduction: Complete or partial loss of chromosome 7 is a recurrent chromosomal aberration frequently found in myeloid disorders such as myelodysplastic

syndromes (MDS) and acute myeloid leukemia (AML). This finding is an important prognostic indicator associated with poor response to the treatment and faster disease progression. However, the exact role of other chromosome 7 aberrations in the pathogenesis of myeloid diseases still needs to be fully understood.
Materials and methods: During 2017-2022, 121 newly diagnosed patients with MDS/AML and chromosome 7 aberrations were examined. Karyotypes were analyzed using a combination of cytogenomic techniques: conventional G-banding, FISH (Abbott, MetaSystems), mFISH/mBAND (MetaSystems), and array CGH/SNP (Illumina, Agilent).
Results: In 101 patients (83%), chromosome 7 aberrations were proved in association with other cytogenomic changes or as a part of complex karyotype, in 17 patients (14%), monosomy 7 was a sole aberration, and three patients (2%) had 7q deletion as the only aberration. The most frequently altered regions were identified in bands 7q11, 7q22, and 7q33-7q36. Deletion of the EZH2 gene in the 7q36.1 region was proved in 50 patients (41%). The most frequently altered regions of the short arm were identified in bands 7p11-7p12. Deletion of the IKZF1 gene in the 7p12.2 region was detected in 21 patients (17%). Rearrangements of 7p were consistently associated with other chromosomal alterations; the isolated 7p aberration was not proved. In 20 patients, structural aberrations of both short and long arm 7p/7q were detected.
Conclusions: We assume that a detailed study of breakpoints and genes located in regions on chromosome 7 can contribute to a better understanding of the molecular mechanisms leading to the onset and progression of the disease and a more accurate diagnosis and determination of prognostic risk in patients with myeloid malignancies.

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P1087 - In multiple myeloma a clear distinction between gain(1q) and amp(1q) is required

Helena Podgornik [1], _Ana Doplihar Kebe [1]_, Hermina Čibej [1], Matevž kerget [1]
University Medical Centre Ljubljana, Department of Haematology, Ljubljana-Slovenia [1]

Multiple myeloma (MM) is a neoplasm with heterogeneous overall survival outcomes (OS). Some IGH translocations (t(4;14), t(14;16), t(14;20)) and del(17p) have been identified as high-risk (HR). Additional copies of the long arm of chromosome 1 (+1q) are among the most common aberrations and occur in 35-40% of newly diagnosed patients (NDMM). +1q can be divided into 2 categories; 3 copies are defined as gain(1q), while at least 4 copies are defined as amp(1q). The impact of +1q on outcomes in MM is conflicting. Although most studies suggest that gain(1q) is of HR, only amp(1q) is uniformly associated with poor survival independent of other cytogenetic abnormalities.
To evaluate the impact of gain(1q) and amp(1q) on OS, we included 136 NDMM. The presence of IGH rearrangements, del(17p), del(1p), +1q, and hyperdiploidy was determined by FISH on isolated plasma cells. OS was analyzed separately for patients with HR cytogenetics, patients with +1q, gain(1q), amp(1q), and patients with other cytogenetic aberrations.
1q+ was detected in 47/135 (35%) of patients, with most (27.9%) having gain(1q) and 6.6% having amp(1q). IGH rearrangements were observed in 32 (23.5%) patients, with prevailing t(11;14) (14%) followed by t(4;14) (6.6%). HR aberrations other than +1q were observed in 16.9% of patients. While t(11;14) and hyperdiploid karyotype were almost mutually exclusive, we could not find any other significant correlation between the studied aberrations. Patients with gain(1q) alone had a similar OS to patients with HR cytogenetics, whereas patients with amp(1q) alone had a significantly worse OS. On multivariate analysis, amp(1q) remained the only statistically significant factor for OS.
Our study was performed in a cohort representative of NDMM, as confirmed by the frequency of prognostically important cytogenetic aberrations, which are in agreement with published data. Although 1q testing was not recommended in the International Myeloma Working Group guidelines, 1q amplification was often classified as HR and is routinely tested in most centers by FISH. Our observation that amp(1q) is a significant marker of poor prognosis supports not only 1q testing but also the clear distinction between amp(1q) and gain(1q).

P1097 - Detection of promoter methylation as well as deletion of MGMT gene in patients with glioblastoma using methodologically different approaches

Halka Lhotska [1], Tatiana Aghova [1], Hana Cechova [2], Libuse Lizcova [1], Karla Svobodova [1], Dora Konecna [3], Petr Hrabal [4], David Netuka [3], Zuzana Zemanova [1]
General University Hospital and First Faculty of Medicine, Charles University In Prague, Center of Oncocytogenomics, Institute of Medical Biochemistry and Laboratory Diagnostics, Prague-Czechia [1] Institute of Hematology and Blood Transfusion, Department of Hla, Prague-Czechia [2] 1st Faculty of Medicine of Charles University and Military University Hospital Prague, Department of Neurosurgery and Neurooncology, Prague-Czechia [3] 1st Faculty of Medicine of Charles University and Military University Hospital Prague, Department of Pathology, Prague-Czechia [4]

Glioblastoma (GBM) is the primary malignant brain tumor with a poor prognosis. The low expression of MGMT gene (localized on 10q26.3), which may be affected by methylation or deletion, is associated with

a good response to temozolomide treatment. However, the appropriate methods of analysis and the cut-off levels for the detection of MGMT gene promoter methylation remain to be discussed.
The brain tissue and peripheral blood of 120 patients with GBM, IDH-wildtype, and WHO grade 4 were investigated. Methylation was analyzed using the MS-MLPA method, ME012 kit (MRC Holland), and Sanger sequencing after bisulfite conversion (Qiagen, Thermofisher Scientific). The deletion of MGMT gene was confirmed by I-FISH (Empire Genomics, Abbott Scientific) and/or CGH/SNP array (Illumina, Agilent).
The methylation of the MGMT gene promoter was confirmed in 38 % of patients. In 80 % of them, the deletion of 10q26.3 region along with the promoter methylation was found. The specificity and sensitivity of MS-MLPA probes were analyzed using the ROC curve approach. The best ratio of sensitivity and specificity has MS-MLPA MGMT_215 probe localized in differentially methylated region 2 (DMR2). The most frequently and intensively methylated was 5´end of MGMT promoter. In 5% of GBM, the methylation of only DMR1 region, which also significantly influences the expression of MGMT gene, was found.
The results of our study show that the MS-MLPA method can reliably detect not only methylation but also deletion of the MGMT gene. The comprehensive analysis of the MGMT gene may lead to more precise and personalized treatment of patients with GBM.
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P1107 - Cytogenetic analysis in acute myeloid leukemia during the 2012 2022 period in Republic of Srpska Bosnia and Hercegovina

Marija Vukovic [1], Svjetlana Stojanovic [1], Mirjana Beric [1], Danijela Mandic [2]
University Clinical Center of Republic of Srpska, Department for Medical Genetics, Banja Luka-Bosnia and Herzegovina [1] University Clinical Center of Republic of Srpska, Department for Hematology, Banja Luka-Bosnia and Herzegovina [2]

Acute myeloid leukemia (AML) is a malignant disease of the bone marrow in which hematopoietic cells are suppressed by intense proliferating myelocytes with malignant transformation. The incidence of AML is 0,9 to 1 per 100 000 inhabitants. In the University Clinical Centre of the Republic of Srpska, for a period of 10 years, 1259 cytogenetic analyses of the bone marrow were performed, of which 107 patients (8,49%) were diagnosed with AML. In the population of Republic of Srpska incidence of AML during 2012-2022 was 0,86%. The most common FAB type of AML was AML-M2 (18,87%), then AML-M4 and AML-M5 (13,2% each), AML-M3 (9,43%), AML-M1 (6,6%) and the least common was AML-M0 with 4,1% of all cases. Unclassified AMLs by FAB classification were present in 33,01% of cases. A normal karyotype was detected in 46 patients (43%). The most frequent chromosome aberration was translocation t(8;21) – at 7,48% of all AML cases. Interstitial deletion of the long arm of chromosome 5 was detected in 5,6%. Deletion 16q22, translocation t(15;17), chromosome aberrations associated with 11q23 region and chromosome aberrations associated with 3q21 region occurred in 4,67% each. Deletion 7q21, deletion 17p, deletion 16q13, deletion 15q15, deletion 9p22 and inversion of chromosome 16 were detected. The following translocations were also detected: t(6;9)(p23q24), t(10;13)(p13;q34), t(17;19)(q21;p13.1) and t(7;17)(p15;p13). Except for structural aberrations, numerical aberrations were detected too: monosomy of chromosome 7 and Y chromosome, as well as trisomy of chromosomes 8, 9, 11, and 13. Cytogenetic analysis has been proven to be necessary to make decisions about further therapeutic protocols.

P1108 - Three paediatric cases of monosomy 7 with different further course of the disease

Pille Tammur [1], Maria Keernik [1], Piret Laidre [2], Sirje Mikkel [3], Lenne-triin Kõrgvee [4], Olga Fjodorova [5], Hanno Roomere [5], Kati Kuuse [5], Tiina Kahre [5], Tiina Kahre [6], Lenne-triin Kõrgvee [7]
Genetics and Personalized Medicine Clinic, Tartu University Hospital, Department of Laboratory Genetics, Tartu-Estonia [1] Genetics and Personalized Medicine Clinic, Tartu University Hospital, Department of Clinical Genetics, Tartu-Estonia [2] Haematology and Oncology Clinic, Tartu University Hospital, Department of Haematology and Bone Marrow Transplantation, Tartu-Estonia [3] Haematology and Oncology Clinic, Tartu University Hospital, Haematology and Oncology Clinic, Tartu-Estonia [4] Genetics and Personalized Medicine Clinic, Department of Laboratory Genetics, Tartu-Estonia [5] Institute of Clinical Medicine, Tartu University, Department of Clinical Genetics, Tartu-Estonia [6] Tartu University Hospital, Tartu University Hospital Cancer Center, Tartu-Estonia [7]

Myelodysplastic syndrome (MDS) in childhood is very rare, one of the most frequent cytogenetic abnormalities detected in paedriatric MDS is monosomy 7 (-7). In our laboratory, 41 cases of monosomy 7 have been identified during 2012-2022, among them only three paediatric cases where additional molecular testing revealed different diagnosis.
PT1. In 4y boy with suspicion of MDS karyotype from bone marrow (BM) revealed 45,XY,-7[20]. Further CMA (Chromosomal Microarray) detected additional changes (del IKZF1 and dup (5)(p15.33)) and the disease was classified as pre-B ALL with other clonal aberration (NOPHO2008 protocol). One year after treatment signs of MDS manifested and secondary AML (treatment related) was diagnosed. Due to accompanying developmental problems clinical exome was performed and pathogenic variant in DNMT3A gene was detected (c.2644C>T, p.Arg882

Cys), Tatton-Brown-Rahman syndrome was confirmed.
PT2. In 2y boy with suspicion of MDS and congenital neutropenia karyotype from BM revealed 45,XY,-7[20]. Subsequent investigation showed normal karyotype (46,XY) from blood. Clinical exome detected previously undescribed de novo variant of uncertain clinical significance (c.4700T>C, p.Ile1567Thr) in SAMD9 gene. Germline mutations of the SAMD9/SAMD9L genes have been found in 17% of paediatric MDS patients, and gain-of-function variants are one of the causes of -7 in the BM.
PT3. In 1y8m boy with haemangioma karyotype from BM revealed 45,XY,-7[20]. Further investigations with TruSight Myeloid next generation sequencing (NGS) panel (Illumina) was performed from BM; clinical exome to detect germline variants was done from buccal cells and TruSight Oncology500 (NGS) to determine somatic variants (Illumina) was made from haemangioma biopsy. All NGS tests detected a pathogenic variant in PTPN11 gene c.227A>G, p.(Glu76Gly) in different levels of mosaicism. In hemangioma biopsy pathogenic variant in GNAQ gene was found: c.626A>C, p.(Gln209Pro), which is often described in congenital hemangiomas. Final diagnosis for PT3 was JMML because it met all the diagnostic criteria for diagnosis as described in the WHO
In summary, the cytogenetic diagnosis of monosomy 7 is challenging, as the course of the disease and the final diagnosis can be vary in wide range, and certainly always requires extensive genome-wide molecular testing.

P1112 - Impact of additional cytogenetics abnormalities in young chronic myeloid leukemia patients single centre experience.

Mehwish Imam Khushk [1], Ikramuddin Ujjan [1]
Liaquat University of Medical and Health Sciences, Department of Pathology, Hyderabad-Pakistan [1]

Chronic myelogenous leukemia (CML) is a hematopoietic disorder of multipotential stem cells, hallmarked by the cytogenetic event t(9;22)(q34;q11), and results in the generation of the Philadelphia (Ph) chromosome carrying BCR-ABL1 fusion gene, which plays a central role in the pathogenesis of CML (1–3). Based on disease course and clinical characteristics, CML is often divided into the relatively indolent, early phase known as Chronic Phase (CP), and the more aggressive advanced phase, consisting of an initial Accelerated Phase (AP) and a fatal Blast Crisis Phase (BC). Imatinib Mesylate (IM), a tyrosine kinase inhibitor, selectively binds and inhibits the tyrosine kinase activity of BCR-ABL1 oncoprotein revolutionizing the survival rate in CML. Survival rates are exceptionally high in CMLCP; however, therapy options for CML-AP and BC are very limited. This may be due to the biological complexity of the disease or the cascade of molecular events responsible for the blastic transformation of CML, which remains inconclusive. The emergence of additional chromosomal abnormalities (ACAs) and other associated genetic defects is a hallmark of multistep disease progression in CML. The most common new aberrations include trisomy 8, an additional Ph translocation, isochromosome 17q, and trisomy 19. The subjects were recruited from the years 2019 - 2022. All 250 study subjects tested positive for the BCR- ABL1 fusion gene, as confirmed by FISH analysis. Cytogenetic analysis was obtained in all but 50 cases (25%) the analysis could not be performed due to the poor morphology of the metaphases. of these 200 cases, ACAs were obtained in 50 patients. The study population consisted of 125 males and 75 females (M: F ratio = 2.1:1) with ages ranging from 17 to 35 years. Trisomy 8, loss of the Y chromosome, low hypodiploidy with 2n = 30–39, and monosomy 17, were also identified in numerical abnormal cases. Out of 50 Cases, 10 patients progressed into the Blast phase. Cytogenetic monitoring is an important pillar of managing patients with chronic myeloid leukemia, especially the young population that cannot be ignored in disease progression and blast transformation

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P1115 - The role of variant t(9;22) in Chronic Myeloid Leukemia A report of 5 cases

Beyhan Durak Aras [1], Sevgi Isik [1], Gulcin Gunden [1], Hava Uskuda Teke [2]
Eskisehir Osmangazi University, Medical Genetics, Eskisehir-Turkiye [1] Eskisehir Osmangazi University, Haematology, Eskisehir-Turkiye [2]

Chronic myeloid leukemia (CML) is a hematological malignancy characterized by the Philadelphia chromosome (Ph). The Ph chromosome is formed as a result of t(9;22) and is detected in 90% of CML patients. However, 15% of the detected Ph chromosomes result from variant t(9;22) (vPh). Variant t(9;22) is grouped as 3-way, 4-way, and 5-way according to the number of chromosomes involved in the translocation in addition to the 9th and 22nd chromosomes.
We detected the vPh chromosome in 5 cases using the conventional cytogenetic method. It was observed that vPH detected in 3 cases had one-step and 3-way mechanisms. The karyotype results detected in the cases are as follows:
Case 1: 46,XY,t(8;9;22)(p21;q34;q11.2)[4]
Case 2: 46,XY,t(1;9;22)(q?;q34;q11)[3]
Case 3: 46,XY,t(9;22;1)(q34;q11;p36.2?)[5]
Case 4: 46,XY,t(9;22)(q34;q11)[3]/46,XY,t(5;9;22;7)(q31;q34;q11;q31)[2]/46,XY[11]
Case 5: 46,XY, t(9;10;22)(q34;q?;q11)[1]46,XY[10]
Although none of the 5 cases we reported had a major molecular response, all were chronic phase CML. In the treatment process, we determined that 3 patients switched from imatinib to nilotinib (cases 1, 3, 5), one patient switched from imatinib to dasatinib (case 2), and the remaining one patient (case 4) continued imatinib treatment.

In the literature, vPh occurring as one-step and 3-way is observed more frequently. In addition, it is generally stated that the variant pH chromosome is not different from the typical t(9;22) clinically and prognostically. However, unresponsiveness to imatinib has been reported in cases. It is noteworthy that our cases also did not respond to imatinib. In addition, the vPH chromosome detected in case 4 has not been reported in the literature before. The patient was treated with imatinib and died at 106 months. As a result, to clarify the prognostic effects of vPH chromosomes, the mechanisms of occurrence of these translocations, and more case report data are needed.

P1117 - Glioma polyploid stem cells are sensitive to the treatments of aurora kinase inhibitor

Martina Giambra [1], Serena Redaelli [1], Alessandra Sirtori [1], Andrea Di Cristofori [1], Carlo Giussani [1], Angela Bentivegna [1]
University of Milano-Bicocca, School of Medicine and Surgery, Monza-Italy [1]

Despite the aggressive standard treatments against glioblastoma (GBM), patients' prognosis is still very poor with a high frequency of relapse. Likely causes for recurrence and treatment failure are the marked heterogeneity of the disease and the presence within the tumour bulk of a small subset of glioma-stem cells (GSC). Nowadays, it has been demonstrated that several tumours, among them GBM, contain polyploid giant cells (PGCs) with stemness properties. PGCs derive from cell fusion or endoreduplication and are characterized by chromosomal instability (CIN). It has also been reported that the number of polypoloid cell and their chromosome content increase after chemo-radiotherapies and PGCs start to exhibit senescence features following treatments. Nevertheless, senescence state is reversible and PCGs may give rise to cells that are drug-resistant and cause tumour relapse. Aurora-serine-threonine kinases are proteins involved in the regulation of the mitotic machinery. Alterations of their genes have been found in a wide range of cancers and have been proposed as attractive PGCs targets since they may be responsible for CIN. Recent data published by our laboratory suggest that a ploidy threshold determines sensitivity of Danusertib, an Aurora kinases inhibitor, in GSC-long-termed cell lines. After treatment, sensitive cell lines showed a senescent/autophagic state following aberrant mitotic exit, while the resistant ones continued to proliferate. Sensitive cell lines presented multi-micronuclei and an enhanced chromosome content than the non-sensitive ones, despite all having missense mutations in TP53 gene. Possible reason for PGC sensitivity of Danusertib may be the variable levels of phosphorylated retinoblastoma (pRb) protein and the expression of the cyclins related to its pathway, such as cyclin-D1, -E1 and –B1. Danusertib can induce apoptosis against melanoma if it occurs after the pharmacological blockage of autophagy. Since TP53 is often mutated in GBM, in this case, apoptosis may occur through activation of E2F1 factor that directly promotes transcription of p53-homologous, p73. This work will deepen the molecular relation between ploidy and senescent/autophagic state after treatment with AurKs inhibitor in order to identify new possible therapies for Glioblastoma.

P1120 - Cytogenetic groups of pediatric acute myeloid leukemia from Ukraine

Liubov Tavokina [1], Uliana Karnaukhova [1], Anna Tavokina [1], Arseny Shustov [1], Natalia Trofimova [1], Nataliia Olkhovych [1]
National Children`s Specialized Hospital "ohmatdyt" Ministry of Health of Ukraine, Department of Oncogenetic Research In Medical Genetics Laboratory, Kyiv-Ukraine [1]

Acute myeloid leukemia (AML) is a heterogeneous group of diseases with specific chromosomal abnormalities (CA), gene mutations, morphology (FAB) and immunophenotype of leukemic cells. CA can be identified in 50-70% of cases. It is customary to divide all patients into cytogenetic groups.
for the period 2020-2022, we examined 71 patients with AML aged from one month to 18 years. Male to female patients had a ratio 60.6% to 39.4%.
Cytogenetic studies were performed on bone marrow aspirate (BM) cells at the initial diagnosis of AML with morphological and immunophenotypic confirmation. We used the standard technique of preparing, cell cultivation, chromosome staining (GTG-bending). FISH was carried out on the nuclei and on metaphase plates using the LSI, TEL, CEP, WCP panel of DNA samples (Vysis Abbott, ZytoVision, Leica Biosystems).
The analysis showed that in 52 (73.2%) patients the karyotype of BM cells was abnormal, while in 19 (26.8%) people it was normal. According to the type of primary recurrent CA in AML abnormal karyotypes were tentatively divided into two subgroups: 1- balanced CA and 2- unbalanced CA. There were 41 (57.7%) and 11 (15.5%) patients in subgroups 1 and 2 respectively. In subgroup 1 typical CA were detected with the frequency:
t(15;17)(q24;q21) – 9.9%,
t(8;21)(q22;q22) – 14%,
inv(16)(p13q22)/t(16;16)(p13;q22) – 5.6%,
rearrangement of the 11q23 /KMT2A locus – 14.1%.
In 2 (2.8%) cases locus 11p15 was involved in t(2;11)(q31;p15), inv(11)(p15q23).
In subgroup 2 we found 4 (5.6%) deletions - (del(5)(q22), del(5)(q33), del(16)(q21), (del(11)(q23 q23) as well as numerical aberrations (+21, +8) in 2 (2,8%) cases.
It is known that CA of each cytogenetic group in combination with the results of other studies are the basis of diagnosis, treatment and have prognostic value. The paper discusses the features of complex diagnostics

and effective algorithms for detection of rearrangements.

P1124 - Glioblastoma from cytogenomics and methylation profile to liquid biopsies and Biomarker Identification

Isabel M Carreira [1], Joana B Melo [1], Domingos Roda [2], Luísa Esteves [1], Gui Rosa [1], Alexandra Mascarenhas [1], Cláudia Pais [1], João Barros [3], Ricardo Velasco [4], Paula Alves [3], Francisco Caramelo [5], Ilda P Ribeiro [1]
University of Coimbra, Cytogenetics and Genomics Laboratory, Coimbra-Portugal [1] Joaquim Chaves Saúde, Algarve Radiation Oncology Unit, Faro-Portugal [2] Francisco Gentil Portuguese Institute for Oncology of Coimbra, Radiation Oncology Department, Coimbra-Portugal [3] Hospital Center Tondela/ Viseu, Neurosurgery Deptarment, Viseu-Portugal [4] University of Coimbra, Laboratory of Biostatistics and Medical Informatics, Coimbra-Portugal [5]

Introduction Glioblastoma multiforme (GBM) is the most aggressive type of primary brain tumors with a limited therapy option. This work aims to characterize through an in-silico pipeline and wet-lab techniques the cytogenomics and methylation landscape of GBM and to explore the utility of cell-free DNA (cfDNA) quantification as a prognostic biomarker.
Methodology Clinical and copy number variations data from 595 GBM patients were retrieved from the TCGA database. Applying a bioinformatic pipeline, the genes and copy number alterations with known oncogenic roles in the most commonly altered chromosomal regions were identified. A validation cohort with 8 Portuguese GBM patients and U87 cell line were analyzed using array Comparative Genomic Hybridization and Methylation-Specific Multiplex Ligation-dependent Probe Amplification, adding conventional and molecular cytogenetics for the cell line characterization. cfDNA from plasma was quantified at four different timepoints during patient's treatment.
Results Besides the identification of known glioblastoma biomarkers, such as amplifications and deletions in chromosomes 7 and 10, where are mapped EGFR and PTEN genes, respectively, several novel genetic alterations were also highlighted, such as amplifications in CPA1/2, CPA4/5, ING3, KLF14 and deletions in BAK1, FADD and MMP21. The most common methylated gene was WT1. Impairment in several oncogenic pathways, PI3K/PTEN/Akt/mTOR and TP53/MDM2/MDM4/CDKN2A-p14ARF were identified. Numerical and structural chromosomal abnormalities were found in the U87 cell line like translocations in the centromere/near-centromeric regions and complex rearrangements involving three or more chromosomes. Preliminary liquid biopsy results revealed that mean cfDNA concentration was higher for patients' pre-maximum safe recession surgery than for controls, which indicate that ctDNA passes through the blood-brain barrier.
Conclusion Our results identify novel chromosomal regions and genes with potential clincal utility.
Longer follow up time is needed for further assess plasma cfDNA as source of prognostic biomarker.

P1128 - Report on the implementation of an early cancer identification and prevention programme among the population of the central Poland

Tadeusz Kałużewski [1], Zofia Gordon-sönmez [2], Marek Stempień [3], Izabela Kubiak [1], Dorota Kucharska [1], Michał Bednarek [1], Jordan Sałamunia [1], Łukasz Kępczyński [4], Tobiasz Kubicki [5], Radzisław Trzciński [6], Anna Bartosińska-dyc [2], Agnieszka Gach [4], Janusz Szemraj [7], Bogdan Kałużewski [1]
Genos Sp. Z O.o., R&d Department, Lodz-Poland [1] Zdunska Wola District Hospital Ltd, Department of Gynaecology & Obstetrics, Zdunska Wola-Poland [2] John Paul Ii Regional Hospital In Belchatow, Department of General and Colorectal Surgery With The Subdepartment of Oncological Surgery, Belchatow-Poland [3] Polish Mother's Memorial Hospital – Research Institute, Department of Genetics, Lodz-Poland [4] Zdunska Wola District Hospital Ltd, Department of Radiology, Zdunska Wola-Poland [5] A Branch of Jan Kochanowski University of Kielce, Collegium Medicum, Piotrkow Trybunalski-Poland [6] Medical University In Lodz, Department of Medical Biochemistry, Lodz-Poland [7]

A population of 1,024 patients were enrolled into the National Cancer Control Programme, which resulted in an identification of 104 cases of germline mutations. The primary objective of the study was to establish an individualised preventive measures and a therapeutic management protocol for the course of breast and ovarian cancer (Module I) and of colorectal and endometrial cancer (Module II). The Module I studies were based on tests for founder mutations in the BRCA1, BRCA2, CHEK2 and PALB2 genes, using the RT-PCR technique and the Sanger sequencing method. In selected cases, the diagnostic procedure was extended by studies of a panel of genes, using the NGS technology. A panel of 70 genes, studied by the NGS and the MLPA technique, was used for the diagnostic objectives in Module II. Following 880 BRCA1 and BRCA2 screening tests, thirty-eight mutations and one mutation were identified respectively. Having applied 843 tests to identify mutations in the CHEK2 and PALB2 genes - nine and three mutations were found, respectively. A targeted family diagnostics identified nineteen mutation carriers in the BRCA1 gene, seven in the BRCA2 gene, two in the CHEK2 gene and three in the PALB2 gene. The panel studies by the NGS method revealed one mutation in the RAD51C gene, one in the CDKN2A gene, one in the CHEK2 gene and two in the PALB2 gene. A total of 144 patients were eligible for the Module II studies. Eleven mutations were identified in the APC gene responsible for familial adenomatous polyposis syndrome, five mutations in

the MLH1 and MSH6 genes responsible for the Lynch syndrome and one mutation in the STK11 gene, responsible for the Peutz-Jeghers syndrome. A complex diagnostic process and a rather sophisticated clinical course of the disease were described in one case, additionally accompanied by the rare NM 007300.4:c.5093_5096del mutation. The description presented here clearly illustrates the role of the time factor, both in the choice of treatment modality and in the diagnostic method implemented.

P1129 - SNP Array characterization of acute lymphoblastic leukemia samples

Joana Barbosa De Melo [1], Luís Miguel Pires [2], Susana Isabel Ferreira [2], Mariana Val [2], Paula Gameiro [3], Ximo Duarte [4], Manuel Brito [5], Isabel Marques Carreira [1]
Laboratório De Citogenética E Genómica – Faculdade De Medicina Da Universidade De Coimbra, Cibb-Centro De Inovação Em Biomedicina E Biotecnologia, Icbr Cimago – Centro Investigação Em Meio Ambiente Genética E Oncobiologia, Fmuc, Coimbra-Portugal [1] Laboratório De Citogenética E Genómica, Faculdade De Medicina Da Universidade De Coimbra, Coimbra-Portugal [2] Laboratório De Hemato-oncologia, Instituto Português De Oncologia De Lisboa, Lisboa-Portugal [3] Serviço De Pediatria E Do Adolescente, Instituto Português De Oncologia De Lisboa, Lisboa-Portugal [4] Oncologia Pediátrica, Centro Hospitalar E Universitario De Coimbra Epe, Hospital Pediátrico, Coimbra-Portugal [5]

Acute lymphoblastic leukemia (ALL) is the most frequent childhood malignancy with an annual incidence that varies between approximately 2-4 cases/100.000 children.
In order to improve survival and quality of survival for children and young adults with ALL, a risk-stratification based on a novel, personalised algorithm using clinical characteristics, genetic changes in the leukemic cells and response to therapy has been developed as stratification strategy of ALLTogether study.
One of the methods that is included for the risk-stratifying genetic aberrations is Array Comparative Genomic Hybridization (aCGH) and Single Nucleotide Polymorphism array (SNPa) to detect the ploidy groups high hyperdiploidy (HeH, 51-65/67 chromosomes), low hypodiploidy (HoL, 30- 39 chromosomes), and near haploidy (NH, 25-29 chromosomes). Because SNPa also identifies uniparental isodisomies it is prefered to either aCGH or conventional karyotype for identifying duplicated clones of NH and HoL, which may be misinterpreted as HeH
In addition, arrays aCGH also allows the identification of intrachromosomal amplification of chromosome 21 (iAMP21) and deletions involving the BTG1, CDKN2A/B, EBF1,ETV6, IKZF1, PAX5, and RB1 genes as well as deletions involving the pseudoautosomal region 1 resulting in rearrangement of the CRLF2 gene.
We analyzed a group of 69 diagnostic ALL samples by SNPa, identifying 23.2% hyperdiploid samples, associated with favorable outcome, 5.8% hypodiploid samples, with a higher percentage than what is reported in the literature and associated with unfavorable outcome, and 71% diploid samples. CDKN2A/B genes deletions were the most frequent, with 11 homozygous and 6 heterozygous deletions identified, and 8 IKZF1 deletions were observed, both imbalances associated with poor prognosis in intermediate risk patients. We only detected one case with iAMP21, also associated with poor prognosis, despite having several cases with trisomy or tetrasomy of chromosome21.
SNPa characterization of diagnostic ALL samples is essential for the identification of the genetic aberrations that impact prognostics and guide therapeutic decisions.

P1140 - Complex translocation leading to13q interstitial deletion in a Moroccan child with retinoblastoma and intellectual disability

Wafaa Jdioui [1], Zhour El Amrani [2], Siham Chafai El Aresearch Team In Genomics and Molelaoui [3], Aziza Sbiti [4], Ilham Ratbi [3], Thomas Liehr [5], Abdelaziz Sefiani [6], Abdelhafid Natiq [3]
Hopital D'enfants, CHU Ibn Sina, Centre Des Consultations Et Des Explorations Externes, Rabat-Morocco [1] Faculty of Medicine and Pharmacy, University Mohammed V/institut National D'hygiène, Research Team In Genomics and Molecular Epidemiology of Genetic Diseases, Genomics Center of Human Pathologies / Département De Génétique Médicale, Rabat-Morocco [2] Faculty of Medicine and Pharmacy, University Mohammed V, Research Team In Genomics and Molecular Epidemiology of Genetic Diseases, Genomics Center of Human Pathologie, Rabat-Morocco [3] Institut National D'Hygiène, Département de Génétique Médicale, Rabat-Morocco [4] Friedrich Schiller University, Institute of Human Genetics, Jena University Hospital, Jena-Germany [5] Faculty of Medicine and Pharmacy, University Mohammed V/Institut National D'hygiène, Research Team In Genomics and Molecular Epidemiology of Genetic Diseases, Genomics Center of Human Pathologie/département De Génétique Médicale, Rabat-Morocco [6]

Background: Retinoblastoma (RB) is the most common malignant intraocular tumor in children; it affects the eyes often even prenatally. RB may be sporadic or familial, due to germinal mutation in RB1 gene or by chromosomal abnormalities involving RB1 gene, located in 13q14. Monosomy of 13q14 as a partial deletion can also be responsible for RB with extraocular symptoms. RB may be associated with psychomotor delay, macrocephaly, and dysmorphic features. Materials and methods: We present here the

case of a patient from a consanguineous family with bilateral retinoblastoma, intellectual disability and facial dysmorphism. Classical and molecular cytogenetics were used to precise genotype-phenotype correlation.
Results: The karyotype showed a complex translocation involving chromosomes 5, 12 and 13. Further molecular cytogenetics analysis revealed a deletion of 13q14 involving the tumor suppressor gene RB1.
Conclusion: This case highlights the impact of classical and molecular cytogenetics in diagnosis of rare genetic syndromes and for the genetic counselling of patients and their families. Pure molecular karyotyping analyses would miss the underlying chromosomal mechanism leading to the rearrangement.

P1141 - Genomic status of PTEN gene and AR expression in primary prostate carcinoma in a cohort of patients from Dobrogea

Anita Cristina Ionescu [1], Mariana Aschie [2], Elena Matei [3], Anca Mitroi [4], Manuela Enciu [5], Gabriela-isabela Baltatescu [6], Nicolae Dobrin [7], Sebastian Topliceanu [8], Georgeta Camelia Cozaru [9]
Institute of Oncology „prof. Dr. Alexandru Trestioreanu" / Medicine Faculty, "ovidius" University of Constanta, Genetics, Bucharest-Romania [1] "sf. Apostol Andrei" Emergency County Hospital / Cedmog "Ovidius" University of Constanta, Pathology, Constanta-Romania [2] Cedmog "ovidius" University of Constanta, Biology, Constanta-Romania [3] "sf. Apostol Andrei" Emergency County Hospital /cedmog "ovidius" University of Constanta, Genetics, Constanta-Romania [4] "sf. Apostol andrei" Emergency County Hospital / Medicine Faculty, "ovidius" University of Constanta, Pathology, Constanta-Romania [5] "sf. Apostol Andrei" Emergency County Hospital / Cedmog, "Ovidius" University of Constanta, Pathology, Constanta-Romania [6] Cedmog, "ovidius" University of Constanta, Electronic Microscopy, Constanta-Romania [7] Cedmog, "ovidius" University of Constanta, Biology, Constanta-Romania [8] "sf. Apostol andrei" Emergency County Hospital /cedmog, "Ovidius" University of Constanta, Genetics, Constanta-Romania [9]

Prostate carcinoma (PCa) is very heterogeneous both in terms of clinical evolution and at the level of genomic changes. Several genomic changes have been identified in PCa, and one of them that may serve as predictors of progression is PTEN gene, a negative regulator of the phosphatidylinositol 3-kinase (PIK3)/AKT survival pathway and a tumor suppressor frequently deleted in PCa. At present, the prognostic value of PTEN genomic deletion is unclear and the androgen receptor (AR) signalling pathway is known to play an important role in PCa.
The aim of the present study was to conduct an analysis of the prognostic and predictive value of AR expression levels and PTEN gene loss in PCa biopsies. Clinical analyses were conducted to determine the associations between PTEN loss and patient outcome. A total of 44 histopathologically proven and diagnosed PCa patients were enrolled in the present study. PTEN gene deletion (using FISH technique) was positiv in 26 patients (59%) while 18 (41%) were negative. PTEN gene deletion was significantly higher in advanced stages as compared to those in early advanced stages. PTEN gene was significantly deleted in patients with the presence of positive lymph nodes compared to patients without positive lymph nodes. IHC staining for AR found significantly lower levels of AR expression within those tumors deleted for PTEN ($P<0.05$).
Conclusion: The present study suggests that PTEN deleted tumors expressing low levels of AR may represent a worse prognostic subset of PCa establishing a challenge for therapeutic management.

P1155 - Laboratory Validation and Clinical Implementation of an RNA sequencing Based Prognostic Assay for Multiple Myeloma

Emily Fullerton [1], Tara Spence [2], Christina Turpin [1], Tamas Revay [1], Amanda Tyndall [1], Dennis Bulman [1], Bob Argiropoulos [1]
Alberta Precision Laboratories, Genetics & Genomics, Calgary-Canada [1] Vancouver General Hospital, Cytogenomics Laboratory, Vancouver-Canada [2]

We describe the laboratory validation and clinical implementation of a targeted RNA-based next-generation sequencing (NGS) prognostic assay for multiple myeloma. The assay employs the Illumina TruSight RNA Fusion NGS panel that targets 507 cancer-associated fusion genes followed by sequencing on the Illumina MiSeq sequencer. Data analysis is performed on BaseSpace (Illumina) using the Illumina DRAGEN RNA Gene Fusion Detection Pipeline to align the reads to the human genome (build GRCh37/hg19) followed by normalization to generate the transcript per million (TPM) values for each target gene of interest. for validation, 100 individual specimens which included both typical and atypical abnormal cases of t(4;14)(p16;q32)-, t(11;14)(q13;q32)- and t(14;16)(q32;q23)- FISH positive cases, were tested. Minimal acceptable aligned coverage for reads in the quality control coverage region of >75% and reads with Q30 bases >80% were required for inclusion in the analysis. Limit of detection studies determined that RNA inputs of 3 ng/µl (≥20 ng) were optimal for high analytical sensitivity which was validated to be 100% for the detection of alterations resulting in elevated expression of the CCND1, FGFR3/WHSC1 and MAF genes. This assay is highly accurate and reproducible. Clinical implementation of the RNAseq assay for multiple myeloma commenced in July 2021 and as of March 2023, 624 clinical samples have been tested and reported. Herein, we describe the validation of an RNA-based NGS clinical test for multiple myeloma and present the findings of nearly two years of clinical implementation. The opportunities and challenges of

using a RNAseq based assay for multiple myeloma will be discussed.

P1156 - Trisomy 14 a rare event in acute myeloid leukemia

Marta Souto [1], Filipa Seixas [2], Ana Matos [1], Catarina Pinto [1], Regina Arantes [1], Manuel Cunha [2], Osvaldo Moutinho [3], <u>Rosário Pinto Leite [1]</u>
Centro Hospitalar Trás-os-montes E Alto Douro, Laboratório De Genética, Vila Real-Portugal [1] Centro Hospitalar Trás-os-montes E Alto Douro, Serviço De Hematologia, Vila Real-Portugal [2] Centro Hospitalar Trás-os-montes E Alto Douro, Departamento Da Mulher E Da Criança, Vila Real-Portugal [3]

Introduction: Trisomy 14 as a sole cytogenetic abnormality is a rare alteration that has been reported in myeloid neoplasms, being myelodysplastic syndrome the most common, followed by myelodysplastic/ myeloproliferative neoplasms and acute myeloid leukemia.
Case Report: An 83-year-old man with history of prostate adenocarcinoma and dementia syndrome, was admitted to Haematology department on December 2022 for pancytopenia, without vitamin iron, B12 or folic acid deficiency and highlight 4% blasts in peripheral blood analyses. Etiological research and medullary examination showed 30% blasts and immunophenotyping classified as acute myeloblastic leukaemia with differentiation. Conventional cytogenetic analysis revealed a trisomy of chromosome 14 in 16 of 20 metaphases. Azicitidine chemotherapy was initiated, but due to the severe toxicity and lack of response it was suspended, remaining currently with hematological support therapy.
Discussion and Conclusion: The present case had a rare trisomy 14 detected by conventional cytogenetics. In the literature, 75 cases of myeloid neoplasms with isolated trisomy 14 have been reported and rarely in acute myeloid leukemia cases. Trisomy 14 was typically found at initial diagnosis, suggesting that this abnormality may represent an early event in leukemogenesis.
The clinicopathologic features and prognostic signifcance of trisomy 14 are not fully understood. However, acute myeloid leukemia with trisomy 14 is often diagnosed in elder males who have an aggressive disease course and a short median survival, ranging from 2 to 12 months.

P1053 - Towards a decision making tool for the identification of chromosome structural abnormalities in conventional cytogenetics Development of a prototype for the detection of del(5q) deletion based on artificial intelligence.

Mohammed El Amine Bechar [1], Jean-marie Guyader [1], Marwa El Bouz [1], Audrey Basinko [2], Nathalie Douet-guilbert [2], Ayman Al Falou1 [1], <u>Marie-bérengère Troadec [2]</u>
Yncréa Ouest, Labisen, Brest-France [1] Chru Brest-univ Brest-inserm 1078, Génétique Chromosomique, Brest-France [2]

Artificial intelligence (AI) is not intended to replace cytogeneticists. However, AI is already positioning itself as one of key supports for medical care. We are developing an innovative AI tool to assist cytogeneticists in detecting structural chromosomal abnormalities from conventional cytogenetic images with diagnostic, prognostic or theranostics objectives in the management of genetic diseases and cancer. More specifically, this tool is developed to verify the correspondence of chromosomal bands between the two chromosomes of a pair. This tool is based on the use of convolutional neural networks (CNN) into an innovative Siamese architecture. As a proof of concept, we focused on the automatic detection of a chromosome structural abnormality such as the deletion of the long arm of a chromosome 5, denoted del(5q), a frequent abnormality in haematological malignancies.
Using our dataset of more than 930 images, we conducted several experiments, without and with data augmentation, in 5 cross-validation tests, on seven CNN models, enabling us to make statistical comparisons for performance assessments. Performances obtained were very significant for the detection of del(5q) deletions for all 7 CNNs. The best CNN achieved sensitivity (detection of deleted chromosomes) and specificity (detection of normal chromosomes) above 99% and 98.5% respectively. As a generalization, these AI architectures were able to successfully recognize another structural abnormality, inv(3). This experience improved when training was applied to the inv(3) dataset with data augmentation, reaching up to 94% sensitivity and 98% specificity. The architecture we propose is the first high performance method based on the Siamese architecture that allows the detection of chromosome structure abnormalities revealed by conventional cytogenetics.

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8. Other Cytogenomic Topics

P1057 - Chromosomal Instability in Mesenchymal Stromal Cells From Acute Myeloid Leukemia Patients

Mateus De Oliveira Lisboa [1], Letícia Fracaro [1], Tamara Borgonovo [2], Valderez Ravaglio Jamur [1], Aline Rangel Pozzo [3], Carmen Lúcia Kuniyoshi Rebelatto [1], Ana Paula De Azambuja [2], Sabine Mai [3], Paulo Roberto Slud Brofman [1]
Pontifícia Universidade Católica Do Paraná, School of Medicine, Curitiba-Brazil [1] Universidade Federal Do Paraná, Hospital De Clínicas, Curitiba-Brazil [2] University of Manitoba, Physiology and Pathophysiology, Winnipeg-Canada [3]

Acute Myeloid Leukemia (AML) is an aggressive clonal disorder of myeloid progenitor cells, in which disease development and progression are related solely by cell-intrinsic mechanisms. However, a single mutation in the bone marrow mesenchymal stromal cells (MSCs) is enough to disrupt normal hematopoiesis leading to leukemogenesis in mice. Remarkably, chromosomal abnormalities (CAs) were found in high frequencies in MSCs from AML patients. CAs in MSCs might indicate Chromosomal Instability (CIN), a phenomenon in which CAs occur at high frequencies. However, the mechanisms that lead to CIN still need to be elucidated. Our study is a comprehensive approach to verify the presence of CIN in MSCs of AML patients. MSCs from AML patients and healthy donors were characterized by conventional cytogenetics (g-banding), fluorescence in situ hybridization (with probes to verify aneuploidy), micronucleus assay, and immunofluorescence (to detect centrosome dysfunction and γ-H2AX foci). The conventional cytogenetics showed a normal karyotype for MSCs from healthy donors, and 71% of the AML patients showed CAs in MSCs. The CAs had a random signature, as previously reported. However, for the first time, we report CIN features such as radial figures, chromatid breaks, polyploid nuclei, micronuclei, chromatid bridge, and centrosome dysfunction. In addition to CAs, these findings support the occurrence of several CIN hallmarks. CAs affect the expression of many genes, which is even more pronounced under CIN conditions. Therefore, CIN might help explain the abnormal gene expression, cytokine production, immunophenotype, and metabolism found in AML-MSCs. Importantly, these abnormalities in MSCs have been associated with the origin, progression, and relapse of AML

P1068 - Molecular Mapping of Two Replication Stress Induced Hotspots of Breakage at the Common Fragile Site FRA11D Harboring Cancer and Neurological Genes

Cynthia Khalil [1], Eliane El Achkar [1]
Balamand University, Biology, Koura-Lebanon [1]

Cancer cells are characterized by their ability to grow at an uncontrolled, quickened and indefinite pace. Point mutations in tumor suppressor genes and proto-oncogenes, changes in chromosome copy numbers and structure are various forms of genome instability that constitute a hallmark of cancer. Several studies suggest a strong correlation and molecular colocalization between breakpoints that lead to chromosome rearrangements in cancer and breaks at nonrandom genomic loci called common fragile sites (CFSs). This finding highlights the importance of CFSs in oncogenesis. CFSs are site-specific breaks that are observed on metaphase chromosomes when cells are cultured under stress conditions. They are found in all individuals and are mainly induced by low doses of aphidicolin, a partial inhibitor of DNA polymerases alpha, delta and epsilon. In this study, our objective is to isolate at the molecular level the hotspots of breakage within the human CFS FRA11D. To induce CFSs, lymphocytes of three healthy donors were treated with aphidicolin at 0.2 and 0.3 µg/ml. Using Fluorescent In Situ Hybridization and Bacterial Artificial Chromosome probes, we located two specific hotspots of breakage within FRA11D. The first is localized at the interface of 11p14.1 and 11p14.2, a result that confirms a previous study that showed that CFSs are present at the junction of late and early replicating bands. The second covers the mucin 15 gene, a member of a family that is widely associated with cancer, a finding that is consistent with the fact that CFSs are associated with cancer genes. In addition, the fragile region and its vicinity contain cancer, neurological and psychiatric genes that are subjected to genomic and epigenetic modifications in several genetic diseases. The molecular detection of these hotspots is an essential step to comprehending the etiology of DNA instability observed in this region in AGR syndrome, autism and haematological neoplasias.

P1074 - CNV Hub a computational tool to classify Copy Number Variations of Unknown Significance using an artificial intelligence based method

Vignesh Guru Pillay [1], Anne-Laure Mosca-Boidron [1], Tristan Moro [1], Gregory Egea [2], Muriel Payet [1], Clémence Ragon [1], Julia Mosnier [1], Alison Bouzenard [1], Dorine Maillet [1], Laurence Faivre [3], Patrick Callier [1], Davide Callegarin [1]
CHU Dijon, Laboratoire de Génétique Chromosomique Et Moléculaire, Dijon-France [1] Genbio-innovie, Laboratoire de Génétique, Clermont-Ferrand-France [2] CHU Dijon, Centre de Génétique, Hôpital D'enfants, Dijon-France [3]

Copy Number Variations (CNVs) are genomic disorders that are common in general population but may sometimes result in human pathologies. CNV interpretation requires multiple parameters to be analyzed and when there is no sufficient pathogenicity evidence, classification as Variant of Unknown Signifi-

cance (VOUS) is inevitable. Classified CNVs are stored on an online database : Cartagenia, that allows us to access data. A periodic reanalysis with updated data is necessary to reclassify all VOUS with new evidence, but is only performed at the request of the clinical geneticist on an individual basis.
Periodic reanalysis of all VOUS from our database is a time-consuming task, however, bioinformatics tools and Artificial Intelligence (AI) assisted programs may provide new solutions. We developed "CNV-HUB", a tool for CNV classification based on recommendations from the French national AChro-Puce consortium. We integrated X-CNV, a CNV analyzing software using AI, in our pipeline in order to optimize it. Then, we developed a first version of CNV-Hub and tested all VOUS from our database. Comparison with other state-of-the-art CNV pathogenicity prediction approaches have been made (X-CNV, AnnotSV, ClassifyCNV and Franklin).
An upstream manual reinterpretation on our VOUS resulted in the reclassification of 5 CNVs as probably pathogenic, and 2 as probably benign. We then tested them on 187 VOUS on CNV-Hub and obtained the same reclassifications for these 7 variants contrary to the other tools. CNV-Hub also classified CNVs related to neurodevelopmental disorders with incomplete penetrance and variable expressivity (PIEV) which is a category of its own as for the AChro-Puce consortium. On the other hand, CNV-Hub wrongly reclassified 57 VOUS as pathogenic or probably pathogenic variations despite having no sufficient evidence for pathogenicity and therefore labelled VOUS after manual reinterpretation.
In conclusion, the first version of our tool, has the advantage of proposing a result given by AI via X-CNV, querying updated databases and classifying variants according to national guidelines from AChro-Puce. CNV-Hub will facilitate the analysis of CNVs and will allow the periodic and systematic reinterpretation of VOUS.

P1101 - Monitoring of long term cultured induced pluripotent stem cells by Optical Genome Mapping (OGM) confirms sustained fine structural genomic stability across more than 60 in vitro passages

Anna Schachner [1], Paul Dremsek [1], Silvia Madritsch [1], Elisabeth Stein [2], Ellen Mosca [2], Thomas Burkard [2], Jürgen Neesen [1], Franco Laccone [1], Jennifer Volz-Glaser [2]
Medical University of Vienna, Institute of Medical Genetics, Vienna-Austria [1] Austrian Academy of Sciences (imba), Institute of Molecular Biotechnology, Vienna-Austria [2]

Genetic integrity testing of induced pluripotent stem cells (iPSCs) is crucial for their safe use in human disease and drug therapy studies. The conventional approach is by karyotyping and genome-wide SNP array, while submicroscopic genetic changes in iPSCs and their dynamics through passaging are poorly documented.
In this study, Optical Genome Mapping (OGM), a next-generation platform for all structural variant types with a 1000-fold resolution vs. karyotyping, was used to track genomic events through early, intermediate and late passages of a fibroblast iPSC line. Concurrently, cells were monitored by karyotyping and long-read sequencing.
Using the Bionano Genomics Gen 2.3 Saphyr instrument, molecules with at least 280 kbp N50 and 300x effective coverage were obtained for de novo-assembly. After removing assembly artefacts from the output, only variants <800 kbp size remained, supporting unremarkable karyograms in all three passages. Variant tracking across passages revealed only minimal changes in the temporal profile of cultured iPSCs. All 24 unique variants, absent in both the Bionano- and our own collective of 180 OGM-characterized genomes, were shared between passages, including one variant of clinical interest.
Of variants not shared between passages, several showed low allele frequencies in single-molecule data, indicating mosaicism as driver of inter-passage variability. However, low-level variants showed a bias towards early passage stages, suggesting that such cell fractions were selected against by in vitro-cultivation. While OGM data can be useful to bridge blind spots in quality control of iPSCs they may require elaborate interpretation strategies complementary to the system´s standard variant calling.

P1110 - Placental activator and inhibitor miRNAs efficiency on abortion development; an epigenetic alteration on true fetal tissues

Elvin Kazancioglu [1], Esra Tug [1], Mehmet Ali Ergun [1], Hasan Huseyin Kazan [2], *Meral Yirmibes Karaoguz [1]*
Gazi University-medical Faculty, Medical Genetics, Ankara-Turkiye [1] Near East University-medical Faculty, Medical Genetics, Nicosia-Cyprus [2]

Background: Small non-coding micro ribonucleic acids, namely miRNAs or MIRs are associated with defective placentation due to their role on the proliferation of the trophoblasts. Relationship between the abortion development and placental defects lead us to search the placental activator (MIR378a-5p, MIR376c, and MIR195) and inhibitor (MIR34a and MIR210) miRNAs expression levels on the accurate pregnancy loss tissues. To this end, XX abortion materials are omitted from the study to exclude the maternal component.
Methods: Total RNA isolation was performed with TRIzol™ reagent directly from frozen trophoblastic tissues belonging to 42 disomic XY, 43 aneuploidic, and 15 triploidic pregnancy loss tissues. All materials were searched for the miRNA expression levels after the mature miRNA levels obtained by reverse transcription via Quantitative Real-Time Polymerase Chain Reaction. This study was supported by the

Scientific Research Fund of Gazi University (Grant TTU-2021-6959) and the preliminary results were presented at the 15th National Congress of Medical Genetics, held on November 2022, at Mugla-Turkey.
Results: The expression of placental inhibitor MIR34a on trisomic trophoblastic tissues was in a high level when compared to the disomic ones (p=0.0324). An increase in MIR195 (p=0.0484) and MIR34a (p= 0.0346) expression levels was observed in numerically abnormal trophoblastic tissues with advanced maternal age group when compared with the disomic ones (with all ages)
Conclusions: It seems likely that the oocyte aging process, which increases with advanced maternal age, contributes to the development of spontaneous abortion with the occurrence of high expression levels of MIR195 and MIR34a. In sum, this study has value to demonstrate the importance of miRNA expression levels in defective placentation and abort pathophysiology as an epigenetic approach, with the necessity of more research on this topic.

P1147 - Clinical Impact of RNA sequencing in Diagnostics

Erika D'haenens [1], Laurenz De Cock [2], Sarah Vergult [2], Lies Vantomme [2], Annelies Dheedene [2], Robin De Putter [1], Tim Van Damme [1], Jo Sourbron [1], Candy Kumps [1], Bert Callewaert [1], Olivier Vanakker [1], Björn Menten [1]
Ghent University Hospital, Center for Medical Genetics Ghent, Ghent-Belgium [1] Ghent University, Department of Biomolecular Medicine, Ghent-Belgium [2]

Background: Several studies have shown that transcriptomics (RNA-seq) nicely complements whole exome and whole genome sequencing (i.e., WES and WGS) in variant interpretation and leads to a 7-36% increase in diagnostic yield. Therefore, we have set up a novel minimally-invasive ready-to-use RNA-seq protocol based on short-cultured peripheral blood monocytes (PBMCs)
Methods: In short, PBMCs are isolated from blood and cultured for 2-3 days. After which the culture is split in two, one of which is treated with cycloheximide to allow detection of nonsense mediated decay sensitive transcripts. Subsequently RNA is extracted and subjected to polyA-sequencing. The RNA-seq data is then processed with OUTRider and/or FRASER or subjected to manual inspection via IGV.
Results: Isolation and culture of PBMCs is faster and less labour intensive in comparison with fibroblasts. Moreover, we show that 63.5% and 62.8% of genes of the Mendeliome (4542 genes) are respectively expressed in PBMCs and fibroblasts, highlighting the potential of PBMCs.
Our workflow revealed an aberrant splicing event in 6/9 individuals with a suspicious splice variant of unknown significance, detected via WES. As these aberrant splicing events were more complicated than anticipated, targeted cDNA sequencing failed to detect the event in 4/6. Based on the RNA-seq results, the six variants could be reclassified and thus lead to a correct clinical diagnosis for these individuals, highlighting the added value of RNA-seq.
Conclusion: We present an optimized RNA sequencing protocol and analysis workflow and show its added value for interpretation and classification of putative splice site variants.

P1151 - Screening of biomarkers for chromosomal instability in the cytogenetic clinic Present status on technological advances and their implementation into routine screening programmes.

Radhia M'Kacher [1], Bruno Colicchio [2], Steffen Junker [3], Kevin Soehnlen [1], Wala Najar [1], Valentine Marquet [4], Claire Borie [5], Andreas Plesch [6], Leonhard Heidingsfelder [6], Noufissa Oudrhiri [5], Nadège Wilhelm-Murer [7], Marguerite Miguet [7], Alain Dieterlen [2], Michael Fenech [8], Annelise Bennaceur-Griscelli [5], Philippe Voisin [1], Patrice Carde [9], Catherine Yardin [4], Eric Jeandidier [7]

Cell Environment, Oncology Section, Evry-Courcouronnes-France [1] Université de Haute-Alsace, Irimas, Institut de Recherche en Informatique, Mathématiques, Automatique Et Signal, Mulhouse-France [2] University of Aarhus, Institute of Biomedicine, Aarhus-Denmark [3] CHU Dupuytren, Service de Cytogénétique, Génétique Médicale, Et Biologie de la Reproduction Hôpital de la Mère et de L'enfant, Limoges-France [4] Hôpital Paul Brousse Université Paris Saclay, Aphp-service D'hématologie-onco-hématologie Moléculaire Et Cytogénétique, Villejuif-France [5] Metasystems Gmbh, Metasystems, Altlussheim-Germany [6] Groupe Hospitalier de la Région de Mulhouse Et Sud Alsace Mulhouse, Service De Génétique, Mulhouse-France [7] University Of South Australia, School of Pharmacy and Medical Sciences, Adelaide-Australia [8] Gustave Roussy Cancer Campus, Department of Hematology, Villejuif-France [9]

Chromosomal instability is considered a robust biomarker for diagnosis of multiple diseases and for prognosis in treatment response and clinical outcome. The aim of this study is to explore if also scoring of micronuclei (MN), nucleoplasm bridges (NPBs), and nuclear buds (NBUDs)can be(1)used as valid additional markers of chromosomal instability in patients with different diseases and (2) implemented as a routine analysis in the clinic.
Materials and Methods: Peripheral blood lymphocytes (PBL) from 200 cancer patients with different hematological malignancies and from 300 patients with reduced fertility were used in this study. PBLs from two hundred healthy donors served as controls. In addition, 20 pathological prenatal samples, 50 cord blood and 20 cell lines established from patients with hematological malignancies were included. Following

telomere and centromere (TC) staining of cytogenetic slides, MN, NPBs and NBUDs were scored. Structural chromosome aberrations were subsequently analyzed by TC+M-FISH to further assess chromosomal instability.
Results: Frequencies of MN, NPBs and NBUDs were significantly increased in patients compared to those in healthy donors. Cytogenetic investigation revealed a significant correlation between loss and deletion of telomeres and the frequency of MN containing TC sequences related to lagging chromosomes in mitosis. NPBs, correlated with the presence of dicentric chromosomes which is a superior biomarker for chromosomal instability. Interestingly, longer NPBs with TC sequences correlated with the presence of dicentric chromosomes with fusion of telomere ends. Shorter NPBs were associated with the presence of a specific configuration of dicentric chromosomes with both centromeres in close proximity. Presence of NPBs in patient samples was related to chromosomal instability and poor clinical outcome.
Conclusion: The results suggest that screening for the presence of MN, NPBs and NBUDs can indeed be employed as valid markers of chromosomal instability. Scoring of these morphological modifications in cytogenetic slides after uniform staining can be subjected to automation thus making the analysis both reliable and fast for stratification of patients without the need for highly skilled

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Read by Title Abstracts

R1 P1014 - Estrogen and progesterone supplementation alters the frequency of cells with 7q deletions in cultured uterine leiomyomas

Alla S. Koltsova [1], Olga A. Efimova [1], Andrei V. Tikhonov [1], Maria I. Yarmolinskaya [1], Vladislav S. Baranov [1], Arina V. Golubeva [2], Dmitrii A. Staroverov [2], Ekaterina D. Trusova [2], Anna A. Pendina [1]
D.O. Ott Research Institute of Obstetrics, Gynecology and Reproductology, Genomic Medicine Department, St. Petersburg-Russia [1] Saint Petersburg State University, Genetics and Biotechnology Department, St. Petersburg-Russia [2]

Literature on Social Media

E.C.A. is now also present on Social Media. Here are announcements of interesting articles that we have posted on Facebook. The articles and news items are related to cytogenomics or to biology in general. If you have relevant articles that you would like to share, please contact mariano.rocchi@uniba.it.

GENETICALLY TRANSITIONAL DISEASE

In an exome screen of 874 genes in 589,306 normal individuals, Chen et al.[1] found 13 adults carrying mutations for 8 severe Mendelian conditions, without any clinical manifestations of the indicated disease. This is just one major example of the pervasive influence of genetic background on both penetrance and expressivity of mutations, indicating that the simplified distinction between monogenic and polygenic Mendelian diseases is inadequate.
In an article in Trends in Genetics, Yao et al.[2] propose using a new term, "Transitional Genetic Disease" (GTD), to describe cases where a mutation that would have a large effect is present but which in itself is insufficient to cause the disease. They suggest that a genetic disease can be considered as the result of gradients of four types of genetic architecture: monogenic, polygenic, GTD, and mixed. They provide examples to explain their proposal which, they conclude, is a preliminary framework that needs input from the medical community and professional societies for further guidance and recommendations.

[1] https://www.nature.com/articles/nbt.3514
[2] https://www.cell.com/trends/genetics/fulltext/S0168-9525(22)00289-X?rss=yes

TRISOMY SYNDROMES

In a paper in Am. J. Hum. Genet. Krivega et al. investigated the physiology of cell lines with different trisomies. Their unprecedented conclusion is that the physiological phenotypes they share could be caused by the presence of an extra chromosome *per se*, regardless of its identity.

[1] https://www.cell.com/ajhg/fulltext/S0002-9297(22)00462-1

THE SEQUENCE AND GENOTYPE VARIATION IN 150,119 GENOMES FROM THE UK BIOBANK

The relationship between sequences and phenotypic variation is usually obtained by exon sequencing, on the assumption that genes are much more important in this matter than the rest of the genome. However, Stefansson et al.[1] (Nature) observe that coding exons represent a small fraction of regions in the genome subject to strong sequence conservation. That means that they are evolutionarily important. The authors analyzed the high-quality whole genome of 150,119 individuals in the UK biobank, and identified 895,055 structural variants and 2,536,688 microsatellites typically excluded from large-scale whole-genome sequencing studies. This analysis discovered "several examples of trait associations for rare variants with large effects not found previously through studies based on whole-exome sequencing and/or imputation".

[1] https://www.nature.com/articles/s41586-022-04965-x

REPRODUCTION - LONGEVITY TRADEOFF

Aging is a hot topic and there are several journals dealing with the physiological and genetic aspects of its progression. However, the full understanding of aging, especially its evolutionary significance, is not fully understood (see previous posts on this topic). The numerous theories on the subject testify to the complexity of the matter. One theory emphasizes the trade-off relationship between reproduction fitness and the aging process. Wu et al. (PNAS) identified, in *Caenorhabditis elegans*, an antagonistic pleiotropic gene, *trl-1*, whose mutants increased brood size but shortened animal lifespan and specifically impaired longevity induced by germline deficiency: a compromise between fitness and longevity. They have not found a corresponding gene in higher organisms, but, they propose, this is a line of research worth exploring.
[1]-
https://www.pnas.org/doi/10.1073/pnas.2120311119?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed

GENES UNDER SELECTION IN CANCER

Technological advances in the analysis of single cells in tumors have made it possible to follow the evolution of cancer by applying concepts and software used in phylogenetic studies (Navin et al. Nature 2011[1]). However, many studies have been hampered by the usual high copy number heterogeneity of tumor cells. In a paper in Genome Research, Hsu et al.[2] propose a general analytic and evolutionary framework for the genotype–phenotype relationship. With this approach

the authors were able to identify genes that had eluded prior genomic searches for positive selection.
They state: “The result … is consistent with the ability of calculus to routinely solve otherwise seemingly intractable problems and is also consistent with the view that biology is likely to follow statistical thermodynamic rules for the large-scale behavior of complex systems that eschews details of internal structures and forces.
Indeed, we find that mutations in genes that are under no specific selective force follow a random distribution. In contrast, genes under selection such as those that drive a group of individuals to a specific location of the fitness landscape will be typified by a non-random distribution. In principle, this is a general approach to identify the genotype determinants of the phenotype specific to a population”.

[1] https://www.nature.com/articles/nature09807
[2] https://genome.cshlp.org/content/32/5/916.long

22q11.2 CNVs IN THE GENERAL POPULATION

22q11.2 is a complex and well-studied region because variations within this domain lead to several pathological conditions, including the most frequent human microdeletion syndrome (22q11.2DS - MIM: 188400), caused by Non-Allelic Homologous Recombination (NAHR) triggered by specific Low Copy Repeats. The phenotypic consequences of this microdeletion have been studied mainly in clinical cohorts. These include cardiac defects, changes in the face and palate, immunodeficiencies, endocrine, genitourinary and gastrointestinal disorders, developmental delay, cognitive deficits, and psychiatric disorders, such as schizophrenia. NAHR also generates duplications of 22q11.2, but phenotypic consequences of these are more elusive, in keeping with the idea that deletions are usually more severe than the corresponding duplications. The fact that these studies were performed on patient cohorts implies that mild phenotypes can escape detection. To overcome these biases, Zamariolli et al.[1] (Am. J. Hum. Genet 2023) analyzed data from 500,000 individuals in the UK biobank to unveil associations of CNV 22q11.2 with traits previously implicated by their genetic content. The study allowed a broad view of the phenotype-genotype correlation of the region under study, in which subclinical manifestations were also highlighted.

[1] https://www.cell.com/ajhg/pdf/S0002-9297(23)00005-8.pdf

ASSOCIATIONS OF PSYCHIATRIC DISORDERS WITH SEX CHROMOSOME ANEUPLOIDIES IN THE DANISH IPSYCH2015 DATASET: A CASE-COHORT STUDY

Most studies of sex chromosome aneuploidies have focused on children and adolescents, and risk estimates for most disorders with typically adult onset have therefore been scarce.
This study (Calle Sánchez et al.[1], Lancet) relies on the iPSYCH2015 case-cohort dataset, based on a source population of individuals born in Denmark between 1981 and 2008 with “case” comprising individuals with a diagnosis of schizophrenia spectrum disorder, bipolar disorder, major depressive disorder, autism spectrum disorder or ADH and “cohort” consisting of individuals randomly selected from the source population.
Analysis of microarray data from bio-banked blood samples of 119 481 individuals (78 726 in the case sample and 43 326 in the cohort) allowed the identification of 387 (0·3%) individuals as carriers of sex chromosome aneuploidies. Each sex chromosome aneuploidy karyotype was associated with an increased risk of at least one index psychiatric disorder and all karyotypes were associated with an increased risk of ADHD, autism spectrum disorder and schizophrenia spectrum disorder. The proportion of sex chromosome aneuploidy carriers who had been clinically diagnosed was 93% for 45,X but lower for 47,XXY (22%), 47,XXX (15%), and 47,XYY (15%).
Increased risks of psychiatric disorders associated with sex chromosome aneuploidies, combined with low rates of clinical diagnosis of sex chromosome aneuploidies, compromise the adequate provision of necessary health care and counselling to affected individuals and their families.

[1] https://www.thelancet.com/journals/lanpsy/article/PIIS2215-0366(23)00004-4/fulltext

WHAT MADE US HUMANS?

In search of fastest-evolved regions of the human genome (Human Ancestor Quickly Evolved Regions, HAQERs).

What is the genetic basis of human uniqueness? The search obviously began with the search for human-specific genes. Genome sequencing of the great apes has revealed, however, that chimpanzees and humans are very similar. As a result, HAQER's research focused on differences in regulatory regions. The search became much more complex when the ENCODE project revealed that regulatory domains are widespread in the genome.
Mangan et al.[1], in a paper on Cell, reported the development of a multiple *in vivo* single cell enhancer assay able to reveal rapid sequence divergence in HAQERs leading to human specific enhancers in the developing cerebral cortex development. They found evidence of both positive selection and high local

mutation rates in HAQERs, once thought to be mutually exclusive, and pointed to an episodic burst of directional positive selection prior to the human-Neanderthal split. They also comment that this high mutation rate is a trade-off between rapid evolution and neurodevelopmental disease.
Trade-off can be considered a general rule in biology. A recent article in Science[2] provided yet another example of a trade-off. B cells express the mutator enzyme activation-induced deaminase (AID), generating double-strand breaks (DSB) at Ig genes through the processing of AID-induced mismatches by DNA repair pathways. In this way a large range of antibodies can be produced. These DSBs, however, can affect proto-oncogenes, especially MYC and BCL6, leading to cancers such as Burkitt's lymphoma and diffuse large B-cell lymphoma.

[1]https://www.cell.com/cell/retrieve/pii/S0092867422013587?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0092867422013587%3Fshowall%3Dtrue
[2] https://www.science.org/doi/10.1126/science.abj5502

KARYOTYPE ABNORMALITIES IN REFERENCE CELL LINES USED IN THE LABORATORY

Established cell lines are very useful because they are immortal and can be used in different laboratories, allowing for cumulative omics data. In this regard, HeLa cells can be considered the first paradigmatic example. They, however, are also a good example of the many chromosomal rearrangements tumor cell lines can contain. Chromosomal rearrangements can also be acquired by normal cells during the immortalization process. This rearrangement can produce dramatic changes in gene expression and affect cell phenotype and behavior during in vitro culture. In an article in Genome Biology, Maslova et al.[1] studied the genomic rearrangements of the chicken LSCC-HD3 cell line (HD3), generated from erythroid precursors and used as an avian model for erythroid differentiation and gene expression. for this task they used the Hi-C methodology[2]. They found that the HD3 cell line has a severely rearranged karyotype with most of the chromosomes involved in translocations. So, it has to be used with caution for erythroid differentiation and gene expression. But, on the other hand, it can be used in studies of genome structure–function relationships.

[1]https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-023-09158-y
[2] https://en.wikipedia.org/wiki/Hi-C_(genomic_analysis_technique)

THE PUZZLE OF AGING

Back to the puzzling topic of aging!
A primary cell culture *in vitro* has a lifespan limit (Hayflick limit). The process towards senescence has been paralleled with aging in vivo.
There are now several exceptions to Hayflick's rule. Soerens et al. (Nature) report yet another. In an immunization, T-cells respond with a burst of cell division. They then become quiescent and represent cellular immunity. After a burst of cell divisions following immunization of mice, the authors transferred the cells into new mice, which then became immunized. They reiterated this experiment 51 times over a period of 10 years. Each time the T-cells responded efficiently. They calculated that in this way the cells were expanding from the starting population by 10^{40} times. Lots to think about in cancer and aging!

[1] https://www.nature.com/articles/s41586-022-05626-9

X-INACTIVATION

It is well known that not all genes on the inactive X are inactivated. San Roman et al. 2023 (D. Page group) analyzed the expression of X-linked genes in lymphoblastoid cell lines and in primary fibroblasts from patients with various X and Y aneuploidies. The analysis revealed an unprecedented and complex situation in which many genes on the active X are modulated in different ways by the inactive X, while the Y chromosome, even with four copies, has little influence. These results may explain, in some cases, the different expression of an X-linked disease in females compared to males.

https://www.sciencedirect.com/science/article/pii/S2666979X23000150?via%3Dihub

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THE MOUSE MODEL CANNOT BE USED FOR SOME FUNCTIONAL STUDIES OF PATHOGENIC GENE VARIANTS ASSOCIATED WITH HUMAN MALE INFERTILITY

Exome sequencing is typically the method of choice for identifying gene variants related to male infertility. Pathogenic variants are then confirmed using functional analysis in model organisms—typically mice. However, the use of the mouse model is not applicable for those genes that are not evolutionarily conserved between mice and humans. In this respect, more than 800 primate-specific genes have been found in the human genome, with a significant proportion of them primarily expressed in the testis (Shao et al 2019; Genome Res. 29, 682–696[1]).
In a recent paper published in the AJHG (Liu et al. 2023; Am J Hum Genet 110:516-530[2]), the authors have used whole-exome sequencing, to identify deleterious variants of X-linked SSX1 in six unrelated

men with asthenoteratozoospermia. Since SSX1 is a gene expressed predominantly in the testis of primates (not in mice), the authors used a non-human primate model (cynomolgus monkey) and tree shrews for the genetic manipulation of SSX1 and its subsequent phenotypic analysis of fertility.
On the one hand, the results obtained in mutant animals (cynomolgus monkey and tree shrews) were consistent with the phenotype observed in humans (reduced sperm motility and abnormal sperm morphology). On the other hand, testis RNA sequencing showed that Ssx1 deficiency influenced multiple biological processes during spermatogenesis. This study provides an alternative strategy for in vivo spermatogenic studies of pathogenic gene variants that cannot be achieved via the murine models. Besides, it also emphasizes the importance of using a multidisciplinary approach to dissect the genetic factors that contribute to male infertility.

[1] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6442393/
[2] https://www.cell.com/ajhg/fulltext/S0002-9297(23)00016-2

GENOMIC MOSAICISM AND DISEASE

Mosacism for aneuploides and DNA variants is quite common in early embryogenesis. Some variants are incompatible with cellular growth and are counterselected. Other variants can persist and can be detected in adult individuals, using deep sequencing. Depending on the extension of mosaicism and the tissue affected, these variants can contribute to an abnormal phenotype.
Truty et al. (2023)[1] analyzed 1 million individuals referred for genetic testing and searched for mosaicism of 1,900 disease-related genes. They found “5,939 mosaic sequence or intragenic copy number variants distributed across 509 genes in nearly 5,700 individuals, constituting approximately 2% of molecular diagnoses in the cohort”.
This large study expands our understanding of the phenotypic implications of mosaicism.

[1] https://www.cell.com/ajhg/fulltext/S0002-9297(23)00083-6

GENOMIC INVERSIONS

The first draft of the human genome sequence was released on 2001. It was ameliorated continuously and in 2013 the hg38 was released. Then the coupling of PacBio and Nanopore sequencing technologies allowed the sequencing of telomere to telomere of all human chromosome, with an incredible precision. This approach can solve the many problems created by repeated sequences, including centromeric and pericentromeric satellites and segmental duplications (SD).
The group of Eichler compared the sequence of 41 genomes against the highly accurate T2T-CHM13 sequence and against the hg38 release (Genom Biol[1]). The comparative analysis was able to detect, among others, novel inversions in the pericentromeric region of chromosomes 1 and 7 (almost inaccessible to classical sequencing), and in SD-rich regions at 15q25.2, 16p11.2, 16q22.1-23.1, and 22q11.21. of note is the fact that the organization of some of these inversions was the minor allele in the hg38.

[1] https://genomebiology.biomedcentral.com/articles/10.1186/s13059-023-02919-8

DECODING THE GENETIC ETIOLOGY OF FEMALE INFERTILITY: INSIGHTS FROM DE NOVO MUTATIONS

Infertility is a widespread issue affecting more than 10% of women of reproductive age. Oocyte maturation arrest and early embryonic arrest are among the leading causes of this phenotype. But, despite its prevalence, the molecular mechanisms that underlie these conditions remain poorly understood.
To address this gap, the authors of this study investigated the role of de novo mutations (DNMs) in female infertility (Li e t al. 2023[1]; Genome Biology 24:68). They analyzed whole exome sequencing data from 473 infertile parent-child trios and identified 481 DNMs, with around 8.32% affecting genes presumed to be involved in female reproductive biological processes. Gene Ontology analysis revealed significant associations with meiosis, embryonic development, and reproductive development. The study focused on TUBA4A, which showed the most significant enrichment of rare DNMs in the infertile trios. Functional assays showed that TUBA4A mutations led to microtubule instability, reduced rates of oocyte maturation, and disruptions in embryo development, mimicking the infertile phenotypes observed in women. The study also identified three other genes (UBQLN1, HTR2C, and ZFPM2) that may play a role in female infertility besides TUBA4A. UBQLN1 is a granulosa cell biomarker for predicting pregnancy in ART, alterations of HTR2C have been associated with implantation failure and pregnancy loss after IVF, and ZFPM2 is necessary for proper fetal ovary development.
This study sheds light on the genetic etiology of female infertility with oocyte and embryo defects and highlights the role of DNMs in human infertility, as demonstrated in other human diseases.

[1] https://genomebiology.biomedcentral.com/articles/10.1186/s13059-023-02894-0

RECOMBINATION BETWEEN ACROCENTRIC CHROMOSOMES

Human acrocentric chromosomes possess distinct traits: (i) in metaphase spreads, they often form satellite associations due to their active involvement of ribosomal DNA in nucleoli formation; (ii) acrocentric chromosomes 13 and 21, as well as 14 and 22, share highly homologous alpha satellite DNA; (iii) on occasion, these chromosomes fuse together to create Robertsonian translocations.
Homologous sequences between centromere and/or short arm are shared by these acrocentric chromosomes and are believed to contribute to the occurrence of heterologous short arm exchanges.
In a recent Nature article, Guarracino et al.[1] took advantage of the recently published T2T sequence of all human chromosomes, to investigate the underlying factors behind these unique characteristics. They discovered “the presence of regions in which most contigs appear nearly identical between heterologous acrocentric chromosomes” and observed a high frequency of heterologous exchanges in these regions. These exchanges provide a comprehensive explanation for the occurrence of Robertsonian fusions, thereby confirming hypotheses proposed by cytogeneticists long ago.

[1] https://www.nature.com/articles/s41586-023-05976-y

PRE-NEOPLASIA (steps preceding cancer)

In his influential depiction of the tree of life, Darwin symbolized the unity of life on Earth by placing a "1" at its base (Darwin, 1859). It implies that all living organisms are subject to the same rules.
Richard Lenski expanded upon this concept with a groundbreaking 1988 experiment, cultivating *Escherichia coli* bacteria for long periods to observe evolutionnary changes across thousands of generations under varying environmental pressures. The extensive research stemming from Lenski’s experiments has significantly advanced our understanding of evolutionnary processes (see “The man who bottled evolution”[1]).
Recent technological advancements have facilitated the study of single-cell 'omics', providing valuable insights into the evolutionary dynamics of cancer (Navin, 2015[2]). However, these studies focus on neoplasias that are beyond the early stages, because pre-neoplasia stages, in humans in particular, are almost inaccessible for experiments. In a Nature paper, Karlsson et al. 2023[3] propose a solution to this challenge, specifically addressing gastric cancer, by employing organoids (gastric organoids, in this case). The suggested solution holds the potential to be a groundbreaking milestone in pre-neoplastic studies, potentially on par with Lenski's revolutionary approach to understanding evolution.
In their experiments they introduced *TP-53* mutations (a common finding in gastric cancer) and monitored the evolution of mutated cells, which resulted in progressive aneuploidy, copy number alterations and structural variants. The study concluded that the earliest stages of tumorigenesis exhibit deterministic evolution and stringent selection (as the title reads), revealing evolutionary constraints and barriers to malignant transformation.

[1] https://www.science.org/doi/10.1126/science.342.6160.790
[2] https://www.nature.com/articles/nature09807
[3] https://www.nature.com/articles/s41586-023-06102-8

ANEUPLOIDIES AND GENE EXPRESSION

Liu et al.[1], in a paper in PNAS, address the problem of gene expression of aneuploidies in different tissues (lymphoblastoid cell lines, fibroblasts and iPSC-derived neuronal cells). They studied 197 individuals with one of 6 sex chromosome dosages (XX, XXX, XY, XXY, XYY, and XXYY). Their conclusion is that dosage effects in *cis* are preserved in the analyzed tissues, whereas trans effects (those on autosomal gene expression) are mostly not preserved.
[1] https://www.pnas.org/doi/10.1073/pnas.2218478120

MOSAIC LOSS OF Y CHROMOSOME IN MICROGLIA AND AGING

Loss of chromosome Y (L0Y) is not a rare event in aging, and has been found to correlate with some age-related diseases, Alzheimer in particular. Vermeulen et al. [1], in a paper in Genome Research, report a large single-cell study of brain cells (851,674 cells) on LOY. Although the frequency of LOY can vary among individuals, there was a consistent enrichment of LOY in microglia with respect to other brain cells, neurons in particular.

[1] https://genome.cshlp.org/content/32/10/1795.long

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Sevilhan ARTAN
Eskisehir Osmangazi University
Medical Faculty
Department of Medical Genetics
Meselik
26480 ESKISEHIR
TURKEY
Tel.: +90 22 22 39 37 71
Fax : +90 22 22 39 29 86
E-mail: sartan@ogu.edu.tr

Joan BLANCO RODRIGUEZ
Unitat de Biologia Cel·lular
Dept de Biologia Cel·lular, de
Fisiologia i d'Immunologia
Facultat de Biociències (Edifici C)
Univ. Autònoma de Barcelona
08193-BELLATERRA SPAIN
Tel. : +34 93 58 13 728
E-mail: joan.blanco@uab.cat

Jean-Michel DUPONT
Laboratoire de Cytogénétique
Hôpitaux Univ. Paris Centre
Hôpital Cochin -
Bât Jean DAUSSET 4e
27 rue du Fbg St Jacquesl
75014 PARIS
FRANCE
Tel.: +33 1 58 41 35 30
Fax : +33 1 58 41 19 95
E-mail.:
jean-michel.dupont@ aphp.fr

José M. GARCIA-SAGREDO
Pabellón Docente, Med. Genetics
Univ. Hospital Ramon y Cajal
Carretera de Colmenar Km 9.100
28034 MADRID
SPAIN
Tel.: +34 91 33 68 550
Fax : +34 91 33 68 545
E-mail:
jgarcias.hrc@salud.madrid.org

J.S. (Pat) HESLOP-HARRISON
Genetics and Genome Biology
University of Leicester
LEICESTER LE1 7RH
UK
Tel.: +44 116 252 5079
Fax.: +44 116 252 2791
E-mail: phh4@le.ac.uk

P.F.R. (Ron) HOCHSTENBACH
Department of Clinical Genetics
Amsterdam UMC
Vrije Universiteit Amsterdam
De Boelelaan 1117
1081 HV AMSTERDAM
THE NETHERLANDS
Tel.: +31 20 44 40 932
E-mail :
p.hochstenbach@amsterdamumc.nl

Thierry LAVABRE-BERTRAND
Laboratoire de Biologie Cellulaire
et Cytogenetique Moleculaire
Faculté de Médecine
Avenue Kennedy
30900 NÎMES
FRANCE
Tel.: +33 4 66 68 42 23
Fax: +33 4 66 68 41 61
E-mail: tlavabre@univ-montp1.fr

Kamlesh MADAN
Dept. of Clinical Genetics
Leiden Univ. Medical Center
P.O.Box 9600
2300 RC LEIDEN
THE NETHERLANDS
Tel.: +31 72 51 28 953
E-mail: k.madan@lumc.nl

Konstantin MILLER
Institut für Humangenetik
Medizinische Hochschule
30623 HANNOVER
GERMANY
Tel.: +49 511 532 6538
E-mail:
miller.konstantin@mh-hannover.de

Felix MITELMAN
Department of Clinical Genetics
University of Lund, BMC C13
22185 LUND
SWEDEN
Tel.: +46 46 17 33 60
Fax: +46 46 13 10 61
E-mail: felix.mitelman@med.lu.se

Maria Rosario PINTO LEITE
Cytogenetics Laboratory
Centro Hospitalar de Trás-os-
Montes e Alto Douro
Av. da Noruega
5000-508 VILA REAL
PORTUGAL
Tel.: +35 1 25 93 00 500
Fax: +35 1 25 93 00 537
E-mail:
mlleite@chtmad.min-saude.pt

Harald RIEDER
Institut fuer Humangenetik und
Anthropologie
Universitaetsstraße 1
40225 DUESSELDORF
GERMANY
Tel.: +49 211 8110689,
Fax : +49 211 8112538
E-mail:
harald.rieder@uni-duesseldorf.de

Mariano ROCCHI
Emeritus Professor
Dip. di Biologia
Campus Universitario
Via Orabona 4
70125 BARI
ITALY
Tel.: +39 080 544 3371
E-mail: mariano.rocchi@uniba.it

Elisabeth SYK LUNDBERG
Dept. of Clinical Genetics
Karolinska Hospital
17176 STOCKHOLM
SWEDEN
Tel.: +46 85 17 75 380
Fax : +46 83 27 734
E-mail:
elisabeth.syk.lundberg@ki.se

Roberta VANNI
Dept. of Biomedical Sciences
Biochemistry, Biology and
Genetics Unit
University of Cagliari
09142 MONSERRATO (CA)
ITALY
Tel.: +39 07 06 75 41 23
Fax : +39 07 06 75 41 19
E-mail: vanni@unica.it

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Treasurer T. Lavabre-Bertrand

ECC SCIENTIFIC PROGRAMME COMMITTEE

Mariano Rocchi (Chair)
Franck Pellestor
Damien Sanlaville
Joris Vermeesch
Emanuela Volpi
Orsetta Zuffardi

E.C.A. News

- The 2023 General Assembly of the E.C.A. with Board elections will take place on 3 July 2023, at 18:30 at Montpellier, France.
- Renewal of the Board in 2023: the following members are due for replacement or re-election in 2023 at the General Assembly: S. Artan (Turkey), Joan Blanco Rodriguez (Spain), R. Hochstenbach (The Netherlands), K. Miller (Germany), F. Mitelman (Sweden).
- Only one list has been received by the President, with the following candidates: Blanco Rodriguez (E), Lindstrand (S), K. Miller (D), Pellestor (F), Yirmibes (TR).

E.C.A. Fellowships

- The **E.C.A.** offers two **Fellowships** for each of the following courses:
  European Advanced Postgraduate Course in Classical and Molecular Cytogenetics
  to be held in Nîmes (France) March 2024 (see pages 92-93)
  Goldrain Course in Clinical Cytogenetics
  to be held in Goldrain Castle (South Tyrol, Italy) 21-29 August 2023 (see page 94)
- The fellowships **include the course fees and the accommodation** during the lectures in Nîmes or in Goldrain but **do not include travel expenses** for either of the courses or for accommodation during the practical training for the Nîmes course. Applications with CV, list of publications and a letter of support should be addressed to the appropriate course organizer. The Educational Advisory Council of the E.C.A. will select the successful candidates.
- **European Cytogenomics Conference 2023** in Montellier: Five poster prizes will be awarded to participants who present the best posters on work derived from a thesis or from a degree awarded between 2021 and 2023. In addition, the E.C.A. offers Fellowships and free registration for early-career researchers presenting posters or talks at the Conference.

MINUTES OF THE E.C.A. BOARD MEETING, MARCH 2023

A meeting of the E.C.A. Board of Directors was held on Friday 24 March 2023 in Hotel Vatel, Nîmes, France

The following 10 board members were present in person: Jean-Michel Dupont (General Secretary), Jose-Miguel Garcia-Sagredo, Pat Heslop-Harrison (Second Vice-President), Thierry Lavabre-Bertrand (Treasurer), Kamlesh Madan (First Vice-President), Konstantin Miller, Maria Rosario Pinto Leite, Mariano Rocchi (President), Elisabeth Syk Lundberg, Roberta Vanni. Present on-line were: Sevilhan Artan, Joan Blanco Rodríguez, P.F.R.(Ron) Hochstenbach, Harald Rieder. Apologies were received from: Felix Mitelman

Opening

The President welcomed the board members and opened the meeting at 17.05.

The Minutes of the E.C.A. Board meeting held on 25th August 2022 in the Goldrain Castle, Italy, and published in Newsletter NL 51, were approved.

Report from General Secretary

The General Secretary reviewed the state of the Membership. The 1267 members comprise 945 members, 20 honorary members, 181 associate members and 121 technologists. The new applications for membership were approved.

New ECA address. As approved at the previous board meeting, the move to the new address and agency has gone ahead successfully. The new official address of E.C.A.: 32 rue Guy Môquet, 92240 Malakoff, FRANCE

Report from the Treasurer

Bank account. Due to administrative complications with the present bank, the board agreed to the proposal that the Association should open a bank account at another bank and maintain two accounts in the future. There will be small costs associated with this.

With low expenditure during the pandemic years, the accounts were in a satisfactory state.

The accounts were approved unanimously by the Board.

Montpellier Conference

The Scientific Program is nearly complete for the 14th European Cytogenomics Conference 1-4 July 2023 in Montpellier, France. The programme was overviewed by The President, and Chairs of the sessions were suggested.

There was some discussion about the Permanent Working Groups.

The Social Programme will be further discussed.

Board elections

The following board members are reaching the end of their period of office: Artan, Blanco Rodriguez, Hochstenbach, Miller, Mitelman. Members S. Artan, F. Mitelman and R. Hochstenbach wish to stand down; the remaining are willing to stand for re-election. The nominations of additional Board Members were discussed, taking into consideration the geographical and topic coverage, and the availability for active participation in the association.

The Board agreed to add the names of Meral Yirmibes Karakoguz, Anna Lindstrand, and Franck Pellestor along with J. Blanco Rodriguez and K. Miller to the list for election at the General Assembly to be held during the ECC14.

Education and courses

The application of scholarships for the Scuola Europea di Cytogenetica – Goldrain were discussed.

The Nîmes course was discussed. There were some issues with visas for students in 2023 and with travel for some lecturers; these were able to join on-line. However, the board considered there was a major advantage in holding the course in person and an on-line alternative should not be formally offered in the future.

The continuing association with the journal Molecular Cytogenetics, with Emanuela Volpi as the new Editor-in-Chief, will be discussed in Montpellier.

The President closed the Board Meeting at 20.10.

Nîmes – France, March 2024

EUROPEAN CYTOGENETICISTS ASSOCIATION (E.C.A.)

European Diploma in Classical and Molecular Cytogenetics

Director: Professor Jean-Michel Dupont, Paris - France

This course was started by Professor Jean Paul Bureau in 1997 and has been held in Nîmes under his directorship until 2017. It is designed to provide advanced training in constitutional, haematological, and oncological cytogenetics to medical graduates, pharmacists, pathologists, biologists, health professionals and researchers, with an academic qualification. The students will be trained to identify genetic abnormalities for diagnosis and prognosis, and for fundamental and applied research using both classical and molecular cytogenetic techniques. The course is co-organized by E.C.A. and two French Universities.

Registration

You can select either

- Basic diploma : only the lectures and a final online examination
- Advanced diploma : same lectures + 2 months training in a cytogenetic laboratory, and onsite final examination in Paris

For registration, please send a letter of application with your CV to the organizers, Prof. Jean-Michel DUPONT (jean-michel.dupont@aphp.fr) or to Prof. Thierry LAVABRE-BERTRAND (thierry.lavabre-bertrand@umontpellier. fr).

The registration fee to be paid by participants was €884 in 2023. For payment by institutions and for more information, please contact the organizers.

Accommodation

A **special** price is available for participants in the 4* Vatel hotel close to the course venue (https://www.hotelvatel.fr/en/nimes) . We highly recommend that all participants stay in this hotel where all the lecturers will be hosted in order to promote interactions during the course.

Scholarships

E.C.A. will award two scholarships covering the registration and accommodation fees. The Education Committee of the E.C.A. will select the suitable candidate.

Scholarship will not be awarded to students whose registration is paid by a third party institution

Topics

Technical Aspects: *Classical Cytogenetics:* Cell culture techniques; Chromosome staining methods (Q-, G-, C-, R- banding and high resolution banding); *Molecular Cytogenetics:* Methods and principles of Fluorescence In Situ Hybridization (FISH) and MFISH; Array CGH; Application of Massively Parallel Sequencing to Cytogenetics; Optical Genome Mapping ; Database use in Cytogenetics; *Laboratory quality assessment.*

Clinical cytogenetics: *Basics:* Frequency of chromosome disorders; Cell cycle, mitosis and meiosis, gametogenesis; Heterochromatic and euchromatic variants; Numerical chromosome abnormalities; Structural abnormalities: translocations, inversions, insertions, deletions, rings, markers; Risk assessment for balanced abnormalities; X inactivation; numerical and structural abnormalities of the X and the Y; Mosaicism; Chimaeras; ISCN 2020; *Clinical:* Phenotype of common autosomal and gonosomal aneuploidies; Chromosome abnormalities in recurrent abortions; Cytogenetics and infertility; Microdeletion syndromes; Uniparental disomy and its consequences; Genomic imprinting; Genetic counselling and ethical issues in cytogenetics; *Prenatal diagnosis:* Indications, methods and interpretation; Risk assessment for chromosomal abnormalities; Non-invasive methods using foetal nucleic acids and foetal cells in maternal blood; Pre-implantation diagnosis; *Cancer* Cytogenetics: Molecular approach to cancer cytogenetics; Predisposition to cancer, Chromosome instability syndromes; Chromosome mutagenesis; Solid tumors; Clinical application in onco-haematology.

Other topics: Genome architecture; Structure of chromatin; Structure of metaphase chromosomes, Mechanisms of chromosome abberations; Origin of aneuploidy; Evolution and plasticity of the human genome; Animal cytogenetics; Plant cytogenetics.

EUROPEAN CYTOGENETICISTS ASSOCIATION (E.C.A.)

European Advanced Postgraduate Course in Classical and Molecular Cytogenetics

Director: Professor Jean-Michel Dupont, Paris – France

The course is scheduled to be held in Nîmes, France March 2024.

2024 Course provisional programme

This approximately 55-hour theoretical part of the course attempts to cover the field of cytogenetics in the broadest sense. The topics can be divided into the following categories:

Technical aspects:

Classical Cytogenetics: Cell culture techniques; Chromosome staining methods (Q-, G-, C-, R-banding and high-resolution banding);

Molecular Cytogenetics: Methods and principles of Fluorescence In Situ Hybridization (FISH) and MFISH; Array CGH; Application of Massively Parallel Sequencing to Cytogenetics; Production and use of molecular probes; Database use in Cytogenetics;

Laboratory quality assessment.

Clinical cytogenetics:

Basics: Frequency of chromosome disorders; Cell cycle, mitosis and meiosis, gametogenesis; Heterochromatic and euchromatic variants; Numerical chromosome abnormalities; Structural abnormalities: translocations, inversions, insertions, deletions, rings, markers; Risk assessment for balanced abnormalities; X inactivation; numerical and structural abnormalities of the X and the Y; Mosaicism; Chimaeras; ISCN 2020.

Clinical: Phenotype of common autosomal and gonosomal aneuploidies; Chromosome abnormalities in recurrent abortions; Cytogenetics and infertility; Microdeletion syndromes; Uniparental disomy and its consequences; Genomic imprinting; Genetic counselling and ethical issues in cytogenetics.

Prenatal diagnosis: Indications, methods and interpretation; Risk assessment for chromosomal abnormalities; Non-invasive methods using foetal nucleic acids and foetal cells in maternal blood; Pre-implantation diagnosis.

Cancer Cytogenetics: Molecular approach to cancer cytogenetics; Predisposition to cancer, Chromosome instability syndromes; Chromosome mutagenesis; Solid tumors; Clinical application in onco-haematology.

Other:

Genome architecture; Structure of chromatin; Structure of metaphase chromosomes, Mechanisms of chromosome abberations; Origin of aneuploidy; Evolution and plasticity of the human genome; Animal cytogenetics; Plant cytogenetics.

16th Goldrain Course in Clinical Cytogenetics

August 22-28, 2023

DIRECTOR

A. Schinzel (Zurich, Switzerland)

PROGRAMME COMMITTEE

A. Schinzel, M.Rocchi, J-M. Dupont, K. Miller, A. Baumer, E. Klopocki

FACULTY

D. Bartholdi (Berne, Switzerland), A. Baumer (Zurich, Switzerland), P. Benn (Farmington CT, U.S.A.), J.M. Dupont (Paris, France), N. Kurtas (Florence, Italy), E. Klopocki (Würzburg, Germany), K. Madan (Leiden, The Netherlands), K. Miller (Hannover, Germany), R. Pfundt (Nijmegen, The Netherlands), G. van Buggenhout (Leuven, Belgium), M. Vismara (Zurich, Switzerland), J. Wisser (Zurich, Switzerland), O. Zuffardi (Pavia, Italy) and others

LOCATION

Goldrain Castle, Goldrain, South Tyrol, Italy

Website of the venue: **www.schloss-goldrain.it**

COURSE DESCRIPTION

The course is focused on phenotypic findings, mechanisms of origin and transmission, correlations of clinical patterns with chromosomal imbalance and modern ways of diagnosis of the latter. Special attention is paid to an understanding how deletions and/or duplications of chromosomal segments cause developmental defects. The course also addresses the optimal application of the diagnostic possibilities, both pre- and postnatally and including molecular cytogenetic methods for a precise determination of segmental aneuploidy.

TOPICS

Dysmorphic findings in chromosome aberrations: formation and interpretation – The adult and elderly patient with a chromosome aberration – Follow-up studies in patients with chromosome aberrations – Clinical findings associated with chromosome aberrations – Microdeletion syndromes: clinical pictures – prenatal cytogenetic diagnosis – Mosaics and chimeras – imprinting and uniparental disomy - Epidemiology of chromosome aberrations – Chromosome aberrations in spontaneous abortions and stillborns – Harmless chromosome aberrations – Risk assessment in structural chromosome aberrations Extra small supernumerary chromosomes – Genomic variation: a continuum from SNPs to chromosome aneuploidy – Pre-implantation cytogenetic diagnosis – Ultrasound findings indicative of chromosome aberrations – Ethical issues in the context of cytogenetic diagnosis – Non-invasive prenatal cytogenetic diagnosis.

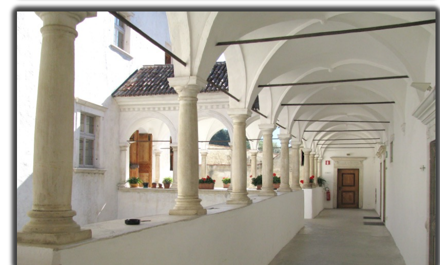
ISCN - Practical exercises in cytogenetic nomenclature – Accreditation of cytogenetic laboratories - Accreditation of cytogenetic laboratories – Optimal use of available techniques in clinical cytogenetics – NGS – SNP arrays and Array-CGH: principles, technical aspects; evaluation of the results – MLPA - QF-PCR - FISH techniques and their interpretation – Introduction and practical exercises with database for phenotypical and variant interpretation - Students presentation of cases with difficult-to-interpret chromosome aberrations. Introduction to modern genetic editing techniques. - Practical exercises will be offered with the ISCN system for chromosome aberrations and with cytogenetic, genomic, and phenotypical databases.

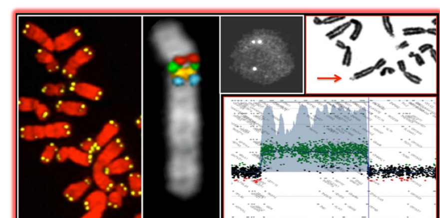
- *Students will have the opportunity to present their own observations and cytogenetic findings which are difficult to interpret, and*
- *they will also have the opportunity to perform a test at the end of the course.*

2022 course

For further questions please write directly to Albert Schinzel at **schinzel@medgen.uzh.ch**

For details: http://www.biologia.uniba.it/SEC/





Full fee is Euro 1600 for a single room or Euro 1450 (VAT included) in a 2-bed-room. It includes tuition, course material, free access to internet during the course, accommodation for 8 nights, all meals, beverages during the breaks and a ½ day excursion.