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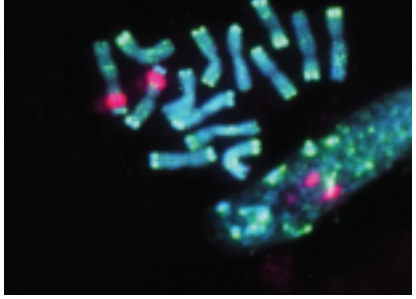
E.C.A. on Facebook

E.C.A. is now also on Social Media! For the present we are active on Facebook, but Instagram and Twitter may follow soon.
Each week 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/**

Please contact us (mariano.rocchi@uniba.it; k.madan@lumc.nl) if you wish to share an interesting news item or a pertinent article.

The Board of the E.C.A. takes this opportunity to wish you all a happy and successful 2020.

European Cytogeneticists Association

12th EUROPEAN CYTOGENOMICS CONFERENCE

6 - 9 JULY 2019

SALZBURG CONGRESS
SALZBURG, AUSTRIA

www.eca2019.com

12th EUROPEAN CYTOGENENOMICS CONFERENCE SALZBURG 2019

Saturday, 6 July 2019

Permanent Working Group (PWG) Reports

PWG: CYTOGENOMICS.

Co-ordinators:
Joris Vermeesch (B)
Anna Lindstrand (S)

The focus of the working group on cytogenomics was structural variation analysis following whole genome sequencing. This is currently a major challenge and likely will be for the coming years. We focused on three topics, callers, databases and visualization.

The first speaker was **Nicole de Leeuw** who presented "Tools for structural variant detection from WGS data. She submitted an overview of different callers and their performance. The bioinformatics team in Radboud University Medical Center has evaluated numerous callers for the detection of structural variation (SV), including deletions, insertions, duplications, inversions and translocations, in the human genome. Four of these were selected for further SV testing using discordant pair-end reads and split reads: Pindel, Delly, Manta and Lumpy. These four callers were compared with each other by using the pilot genome from Genome In A Bottle (GIAB), looking at sensitivity, specificity and running time. Manta yielded the best test results and is currently used together with Control-FREEC for CNV calling and ExpansionHunter for the detection of short tandem repeats.

The second speaker was **Jesper Eisfeldt** who presented "Structural variation databases based on whole genome sequencing data". Jesper presented an overview of gnomAD-SV and SweGen-SVDB, two public structural variant frequency databases, as well as strategies for building local (private) structural variant databases. These databases were compared based on the number of filtered common variants in a cohort of 100 individuals, illustrating that local databases are necessary for filtering local technical artefacts as well as population specific variation.

The third speaker was **Johanna Lundin** who presented "VCF2Cytosure", a new tool for visualization of structural variants developed by researchers at the Karolinska Institute together with the Dept of Clinical Genetics at the Karolinska University Hospital. This tool converts VCF-files from whole genome sequencing into CGH-files suitable for uploading in the Cytosure Interpret software (Oxford Gene Technologies). In this software the structural variants are visualized in the same way as CGH data and can be analyzed and classified in a very user-friendly way suitable for a clinical setting.

PWG: CYTOGENETICS OF HAEMATOLOGICAL MALIGNANCIES.

Co-ordinators:
Bertil Johansson (S)
Harald Rieder (D)

About 50 participants joined the PWG meeting.

The meeting started with the presentation of an abstract entitled "A unique peripheral blood karyotype characterized by multiple complex chromosomal translocations is caused by homozygosity for the CHEK2 p.Gly167Arg variant" which was given by **Nivin Moustafa** (IL). She reported about two patients who were unrelated and were homozygous for the CHEK2 c.499G>A; p.Gly167Arg variant, located in the central forkhead-associated (FHA) domain of the protein. One patient presented with primary

multi-organ tumorigenesis including dozens of intestinal polyps since age 35 years, thymoma at age 49 years, breast cancer (BC) at 65 years, prostate cancer at 66 years, left renal cell carcinoma, angiomyolipoma of the right kidney and sigmoid gastrointestinal stromal tumor at age 67 years. Karyotyping on peripheral blood lymphocytes revealed multiple different non-clonal chromosomal translocations involving various chromosomes in 40%–60% of cells, which were mostly unbalanced. The patient's bone marrow and fibroblasts were normal and did not display the translocation phenotype. The second patient had early-onset acute myeloid leukemia, which was characterized by a clonal karyotype including a t(3;8)(q26;q24) in the bone marrow cells. The chromosome analysis of peripheral blood cells showed different non-clonal complex chromosome aberrations in about 30% of the cells. It was discussed that the homozygosity for p.Gly167Arg in CHEK2 increases the patients' susceptibility to DNA double strand breaks (DSB) and shifts the balance to non-accurate DSB correction. This possibly explains the increased susceptibility of homozygotes to either multiple primary tumours during their lifetime or early-onset tumorigenesis.

Oskar Haas (A) presented the paper entitled "Hyperdiploid acute lymphoblastic leukemia with genome-wide copy-neutral loss of homozygosity (CN-LOH)". He addressed a distinct subset of hyperdiploid ALL in children, which is characterized by pure tetrasomies. Such cases show a CN-LOH of all disomic as well as the duplication of both homologs of all tetrasomic chromosomes. It is generally believed that this distinct pattern can only derive from the duplication of a preexistent analogous hyper-haploid clone. As apparent indicator of poor prognosis, such hyperhaploid/-diploid ALL forms are nowadays stratified as high risk in ongoing treatment protocols. Oskar Haas showed, that hyperhaploid and analogous hyperdiploid forms cannot be distinguished based on array patterns alone. Therefore, he considered it essential and mandatory to use cytogenetic, FISH and/or DNA index analyses in addition to microarray investigations to clearly assess the pattern of the chromosomal gains and losses more accurately.

Harald Rieder (G) presented the abstract "Interactive online karyotyping to teach cyto-genetics in undergraduate medical education". The online karyotyping tool was available for the participants of the PWG meeting by using their own device. Chromosomes could be dragged by mouse or pencil to complete a karyogram. If a karyogram was completed it was submitted to the system to check for mistakes. In case of mistakes the system rejected the karyogram until it was corrected. After the completion of the karyogram, a karyotype according to the ISCN had to be provided which again was checked by the system. A total of seven different chromosome aberrations were included in the online tool and successfully used for teaching cytogenetics in undergraduate medical education. The meeting closed with several participants staying on to continue with online karyotyping.

PWG: CANCER CYTOGENOMICS, SOLID TUMOR STUDIES.

Co-ordinators:
Roberta Vanni (I)
David Gisselsson-Nord (S)

The Permanent Working Group (PWG) on "Cancer Cytogenetics, solid tumor studies" met in Salzburg on July 6, 2019 during the 12th European Cytogenomics Conference.

The aim of the meeting was to define the "State of the Art Seminar on Solid Tumor Cyto-genomics".

The Co- coordinator **Roberta Vanni** started the session by thanking the presenters of the selected abstracts - who had enthusiastically accepted to share their results with the community - and gave an overview of the development of the research in solid tumor cytogenetics. The emerging evolution of this field in the last years has paralleled the tremendous evolution of new molecular techniques, most prominently the next generation (massively parallel) sequencing. This trend was reflected in part by the abstracts

presented during the PWG. In line with this trend and the new title of the European ECA Conference (European Cytogenomics Conference), the participants agreed with the proposal to change the title of the Permanent Working Group to "Cancer Cytogenomics, solid tumor Studies".

The co-coordinator **David Gisselsson** introduced the speakers and moderated the discussion.

The first speaker, **Dr. Alla S Koltsova** from St. Petersburg State University, reported a study on cytogenetic abnormalities in uterine leiomyoma cells in which the frequency of karyotypically abnormal clones *in vivo* and *in vitro* was compared. The authors observed a statistically significant difference in frequency of abnormal cells between cultured and non-cultured samples and suggested that some of the abnormal clones (but not all) have a selective growth, in contrast to cells with chromothripsis which were less represented in cultured cells.

The second speaker, **Dr. Alvin Soon Tiong Lim**, from the Singapore General Hospital, Molecular Pathology, reported on the diagnostic role of MAML2 gene rearrangements, disclosed by FISH, in mucoepidermoid carcinoma (MEC) and highlighted that MAML2 testing is needed for tumors deviating from the conventional appearance of MEC and those resembling other tumor types.

The third speaker, **Dr. Aakila Sammy**, from the Brunel University London, discussed the results of a research on ovarian cancer analytic cytogenomics. Her group had investigated mis-localization and re-organization of specific ovarian cancer-related chromosomes and genes by analyzing nuclear motor myosin, which has a role in the adaptation of chromosome territories under different physiological conditions. Knocking down the protein, they compared the response of ovarian cancer cells, healthy ovarian cells and cells characterized by drug resistance. They concluded that genome organization represents an exploitable mechanism in which the protein may play a significant diagnostic and prognostic role.

Last but not least, **Dr. Eric Jeandidier**, of the Groupe Hospitalier de la Région de Mulhouse et Sud-Alsace Service de Génétique Mulhouse-France, reported on how they improved the detection of dicentric chromosomes and telomere dysfunction, the driving forces of chromosomal instability. Their approach consisted of sequential analysis of telomeres and centromeres using FISH followed by the M-FISH technique. This allowed the assessment of the potential role of telomere dysfunction and chromosomal instability in order to improve initial treatment strategy on an individual basis.

PWG: QUALITY ISSUES AND TRAINING IN CYTOGENETICS.

Co-ordinators:
Ros Hastings (UK),
MartineDoco-Fenzy (F)
Marta Rodríguez de Alba (E)

On behalf of the PWG on Quality and Training, Dr Ros Hastings presented a workshop on ISCN. The talk started by giving some of the common misconceptions, then detailing some of the basic ISCN rules before giving examples of frequent errors seen in the GenQA External Quality Assessments (EQA). Finally there was an interactive ISCN quiz for everyone to participate in.

Ros represents Europe on the Standing Committee for ISCN and at a recent committee meeting it was decided there would be a major revision with a new ISCN being published in 2020. The committee reviewed 222 suggestions for improvement and agreed to include some of the suggestions. The committee agreed there was a need for some triple-colour FISH examples plus more examples were needed in the rsa and sequence chapters. In addition, it was proposed that the chapter on comparative genomic hybridisation (13.6) could be removed and that section 8.4 on UPD should be moved to the array

chapter. Finally, it was proposed that a new chapter for Polar Body ISCN to describe haploid sets should be included.
It is also proposed that the new ISCN 2020 will include the following changes:

- A summary of the basic ISCN rules will be given at front of book.
- Sex chromosomes will be reported first for all techniques.
- Abnormalities will be described from pter to qter for all techniques so that the G-banded nomenclature is consistent with the 'arr' and 'seq' nomenclature.
- Nucleotides can be separated by commas (but not full stops) for arrays, rsa and sequence nomenclature.
- When an unbalanced derivative is inherited from a balanced rearrangement in the parent 'dmat' and 'dpat' will be used. The previous use of mat or pat for an unbalanced derivative is ambiguous as could also imply the parent is also unbalanced.
- Centres will have to specify which HGVS version is being used for 'seq' nomenclature.
- More p-arm examples will be given.

A pdf copy of the talk can be found on the GenQA website for any interested genetics centres on https://www.genqa.org/sites/default/files/PWG%20-%20ECA%202019.pdf
Dr Ros Hastings has now stepped down from this PWG as she has retired and 'returned' to work part time with GenQA. Thanks to all the ECA members who have supported this PWG at the ECA conferences over the years. I hope you will continue to do so under the able leadership of Dr Martine Doco-Fenzy who will now take the lead on this PWG.

Ros Hastings
Outgoing Chair of the PWG for Quality and Training.

The Board of the E.C.A. is grateful to Ros Hastings for her excellent work as a coordinator of this PWG for many years.

PWG: MARKER CHROMOSOMES

Coordinators:
Thomas Liehr (D)
Isabel Marques Carreira (P)

As is customary, the permanent working group meeting, PWG Marker Chromosomes was held on the first day of the 12th ECA-conference 2019 in Salzburg, Austria. This was the 7th meeting of the PWG. The session was again well appreciated and attended by >150 cytogeneticists from all over Europe.
Thomas Liehr (Jena, Germany) briefly introduced the topic of this specific PWG and outlined the programme. Five speakers, who had been selected from the abstracts submitted to the conference, gave a 5-8 minute presentation on small supernumerary marker chromosomes (sSMC) identified during routine diagnostics. **Wafa Slimani** presented 8 out of 33 sSMC cases characterized in her lab in Sousse (Tunisia) during the past 8 years: six cases of sSMC(15) and 2 cases with partial tri- or tetrasomy 9p; these were also discussed in the context of available literature. **Nadezda Shilova** talked about a man with the karyotype 47,XY,+invdup(22)(p11.2) 10]/ 46,XY [20] on whom extensive studies on meiotic segregation of this sSMC had been performed in their Moscow lab (Russia). As a result, they could show that the presence of this sSMC had no influence on the rate of aneuploidy in the sperm of this healthy sSMC carrier. The next presentation, by **Jadranka Vraneković** (Rijeka, Croatia), showed the difficulties of diagnosing a prenatal case of a Pallister-Killian syndrome with a unique feature, a bifid cardiac apex which has never been described before. **Yvonne Stratis** (Mainz, Germany) reported the first sSMC with a neocentromere resulting from a complex chromosomal rearrangement involving an insertion of chromosome-4 material into a chromosome 12, and a deletion of a part of chromosome 12. The deleted segment 12q2?3~q24.33 formed an sSMC. **Paolo Reho** (Florence, Italy) showed how low coverage whole genome sequencing in plasma-derived circulating cell free DNA can be used to detect low levels of

sSMC(X) or sSMC(Y) in cases of Turner syndrome, who were previously thought to have a karyotype 45,X in all cells.
Finally, **Thomas Liehr** (Jena, Germany) reported on the next project to be done in close cooperation with FACE2GENE and on, thanks to a financial support of NORD foundation, the establishment of computer-assisted recognition of patients with cat-eye-syndrome. This has been already implementted and published for Pallister-Killian and for Emanuel syndromes (PMID: 28661575). Just like the other speakers in this session, Dr. Liehr also emphasized the irreplaceable impact of (molecular) cytogenetics in a world of array-CGH and NGS; he referred to a recently published paper of his group (PMID: 30089300) in which it was shown that >80% of sSMC carriers would be missed if cytogenetics would be skipped and be replaced by array-CGH in the diagnosis of infertility. Finally, problems with maintenance and updating of the well-appreciated sSMC page (http://ssmc-tl.com/Start. html) and its sister pages (http://ssmc-tl.com/Start. html) were outlined. These problems will mean that sooner or later in 2019/2020 this page can no longer be accessed via that link. Just in case this issue cannot be solved, the page is already now available on http://molbiol.sci.am/ssmc/ssmc-tl.com/Start. html. Dr. Liehr is working on a new version and will announce where it can be found - this will be possible via the last mentioned link or via http://markerchromosomes.ag.vu, http://markerchromosomes.wg.am or https://www.uniklinikum-jena.de/humangenetik/en/Databases.html.

Overall, the meeting showed that there is a broad spectrum of different sSMCs, which can be detected in our routine pre- and postnatal studies. Thanks to all speakers for giving their excellent presentations, and it was really a pity that **Isabel Marques Carreira** (Coimbra, Portugal) could not come in time to the meeting due to travel problems!

PWG: PRENATAL DIAGNOSIS.

Co-ordinators:
Maria Rosario Pinto Leite (P)
Jean-Michel Dupont (F)

A meeting of the permanent working group on Prenatal Diagnosis was held on 6 July during the 12th European Cytogenetic Conference 2019 in Salzburg. Approximately 80 participants attended the session.
There were three presentations, including one last minute abstract on Uniparental Disomy risk estimate.
Rosário Pinto Leite and **Jean Michel Dupont** challenged the audience to take part in an innovating and dynamic experiment during the presentation: answering in real time a survey (quiz) about practices in CVS in Europe. The answers provided to each question were then compared with previous responses given by colleagues from several countries, including several laboratories in Portugal. This survey, of which results will be available later on, gives a first glimpse of the heterogeneous practices all over Europe with regard to CVS handling.
The presentation by **Aurélie Coussement**, from the Laboratoire de Cytogénétique, Cochin hospital in Paris, was titled “Back for the future - Lessons from the past for an updated management of the trophoblast”. It focused on the management of chorionic villus samples for chromosome analysis, starting in the 80s where only direct analysis was available from the cyto-trophoblast, to the presentday protocols including arrayCGH. She presented the pros and cons of the successive strategies used and showed that the association of direct analysis on cytotrophoblast associated with either FISH on mesenchymal cells (in case of aneuploidy) or array CGH in case of normal result give the best compromise between time to get a result and sensitivity.
Kamran Moradkhani, from Service de Génétique, CHU Nantes, presented “Risk estimation of uniparental disomy of chromosome 14 or 15 in a fetus with a parent carrying a non-homologous Robertsonian translocation”. In accordance with the results of this study, the authors do not

recommend prenatal testing for UPD for pregnancies when one of the parents is known to carry a nonhomologous ROB involving chromosome 14 and/or 15. A total of 1747 UPD testing were performed on fetuses during pregnancy for the presence of UPD(14) and/or UPD(15); the risk of UPD following prenatal diagnosis was estimated to be around 0.06 %, less than the risk of miscarriage following an invasive prenatal sampling. This study was published in July 2019 in Prenatal diagnosis (https://doi.org/10.1002/pd.5518)

PWG: CYTOGENETIC TOXICOLOGY AND MUTAGENESIS.

Co-ordinators:
José M. Garcia-Sagredo (E),
Emanuela VOLPI (UK)

The meeting of the Cytogenetic Toxicology and Mutagenesis PWG was held in the Salzburg Conference Centre on the first day of the 12th ECA Conference. The session was opened and chaired by the PWG Coordinators, **Emanuela Volpi** (University of Westminster, London) and **José Garcia-Sagredo** (Alcala' University). The central theme for this year's meeting was *the evaluation of chromosomal instability applied to diagnosis, prognosis and treatment of disease*.

from left to right: Jose Garcia-Sagredo, Ulrike Mau-Holtzmann, Emanuela Volpi, Isadora May Vaz, Radhia M'kacher and Eric Jeandidier.

The first two invited talks focused on chromosomal instability in cultured stem cells and its relevance in connection to the safe use of these cells for clinical applications.

The first speaker - **Isadora May Vaz** from the Pontifícia Universidade Católica do Paraná in Curitiba in Brazil - showed how the reprogramming and *in vitro* culture of induced pluripotent stem cells (iPSC) from karyotypically healthy mesenchymal cells can lead to a completely altered lineage through the emergence of clonal cytogenetic changes (Abstract 1131).

In line with the first talk, in her presentation titled 'Quality control: CHECK YOUR CULTURES! Karyotyping identifies genetic instability in iPSC' (Abstract 1157), the second speaker – **Ulrike A. Mau-Holzmann** from the University of Tuebingen in Germany – emphasized 'chromosomal instability' and the resulting gain of chromosomal aberrations during reprogramming, transdifferentiation or gene-editing as common features during iPSC culturing, and advocated periodical karyotyping as an effective quality control measure. A lively discussion ensued leading to a consensus on the necessity to develop means to increase awareness of this issue with the relevant communities of stem cells practitioners.

The next two talks were delivered by two collaborating colleagues from France, **Radhia M'kacher** (Cell Environment DNA damage R&D, Paris) and **Eric Jeandidier** (Groupe Hospitalier de la Région de Mulhouse et Sud-Alsace). Eric (Abstract 1202) presented development of a protocol based on centromere and telomere staining followed by M-FISH for the detection of dicentric chromosomes and the assessment of telomere dysfunction in connection with chromosomal instability in patients with haematopoietic malignancies. Radhia (Abstract 1200) reported their interesting results on the identification of telomere dysfunction and pericentromeric breakpoints in patients undergoing fertility treatment suggesting telomere status as a potential novel biomarker for the prediction of outcome in assisted reproduction.

The final talk was given by **Ivan Iourov** (Mental Health Research Centre, Moscow, Russia). Ivan presented the most recent data by Yurov's team on their longstanding project on somatic chromosomal mosaicism and instability in neurodevelopmental diseases (Abstract 1097), with novel empirical evidence, which is bound to generate renewed interest in this fascinating and challenging research area.

It has been an honor and a pleasure to organize this PWG meeting and to have the opportunity to meet talented, like-minded colleagues from different parts of the world to discuss research topics of common interest. We very much look forward to the next PWG meeting.

PWG: CLINICAL AND MOLECULAR APPROACHES TO CYTOGENETIC SYNDROMES

Co-ordinators:
Conny van Ravenswaaij (NL),
Cristina Skrypnyk (BRN),
Nicole de Leeuw (NL)

During this one-hour PWG meeting, **Nicole de Leeuw** started with an update on ECARUCA and the transition to DECIPHER to enable the continued sharing of the gathered content of the ECARUCA database, which contains detailed, curated clinical and molecular information from more than 5,000 patients with a rare, unbalanced chromosome aberration. A total of 3,646 patients with 4,365 genomic imbalances qualify to be submitted to DECIPHER. ECARUCA account holders who submitted cases in the past 15 years will be contacted to obtain adequate consent for submission in DECIPHER.

Next, selected abstracts from three ECA participants were presented, the first two focussing on the 3p26.3 region. Both copy number gains and losses in the 3p26.3 region have been associated with neurodevelopmental disorders. **Andreea Cristina Stanciu** from the University of Medicine and Pharmacy in Bucharest, Romania, gave a presentation entitled "The first case of 3p26.3 deletion containing only *CHL1* gene associated with ASD". She gave a brief summary on the 3p deletion syndrome (OMIM #613792) which is a rare contiguous gene disorder caused by deletions in the distal 3pter region. It is characterized by psychomotor retardation, developmental delay, dysmorphisms, microcephaly and ptosis. Only four well documented cases have been reported so far, each with a terminal deletion in 3p26.3 (500 kb - 1.1 Mb) encompassing only the *CHL1* gene. Three of these were inherited from an apparently unaffected parent. *CHL1* is highly expressed in the central and peripheral nervous systems and copy number variants involving this gene have been considered causative for impaired cognitive function. Andreea presented a 12-year-old boy with mild intellectual disability, speech delay, motor delay and hyperkinesia in whom array-CGH analysis disclosed an interstitial loss of 12 kb in 3p26.3 causing a partial deletion of the *CHL1* gene. Unfortunately, carrier testing in the parents was not (yet) performed.

Next, **Igor Lebedev** from the Research Institute of Medical Genetics in Tomsk, Russia, presented their work on "*CNTN6* expression in human IPSC-derived neurons from a patient with neurodevelopmental disorder and 3p26.3 microduplication and the same microduplication healthy carrier". This duplication was previously characterised by whole genome sequencing, which did not reveal any structural variations either within the *CNTN6* gene or its flanking regions. They next obtained cell lines of induced pluripotent stem cells (iPSC) from cells of the patient and his unaffected father carrying the same duplication (of maternal origin in the father). The iPSCs were differentiated in vitro into cortical neurons. The level of *CNTN6* gene expression in patient iPSCs was significantly lower than in the neurons from two healthy donors (without the duplication). The level of *CNTN6* expression in the carrier father iPSCs was comparable to that in neurons from the healthy donors. Allele-specific analysis of *CNTN6* expression revealed slightly preferred expression of the maternal allele in control iPSCs. This preference was more pronounced in

the father carrying a maternal duplication. The expression of the duplicated allele of paternal origin in the patient was significantly weaker than a normal one which caused an even more profound difference between maternal and paternal allele expression of *CNTN6*. The significant reduction of the *CNTN6* expression in neurons obtained from patient iPSCs can explain the similarity of the symptoms observed in patients with either a deletion or a duplication of the *CNTN6* gene.

Ana Sousa (CHULN-HSM, EPE Medical Genetics Department in Lisbon, Portugal) was the last speaker in this meeting and she talked about "*STAG1* haploinsufficiency: an emerging phenotype". She presented data on a 4-year-old boy with mild developmental delay, arched and sparse eyebrows, down-slanting palpebral fissures, a wide nose with short columella, and thick lips. An intragenic deletion of 206 kb, involving exons 2 to 12 of the *STAG1* gene (arr[GRCh 37]3q22.3(136184662_136390897)x1), was detected by array CGH analysis. *STAG1* encodes one of the components of the cohesion complex which is involved in chromosome segregation and gene transcriptional regulation. *STAG1* is one of eight genes participating in the cohesion pathway. A dysfunction in this pathway my lead to one of the cohesinopathies, which are rare neurodevelopmental disorders characterized by distinctive facial dysmorphism, growth retardation, developmental delay/intellectual disability (DD/ID), and limb abnormalities. So far, only 15 patients with a (partial) loss of *STAG1* or a pathogenic nucleotide variant in *STAG1* have been reported in the literature. The patient presented here shares common clinical features with these published patients and these features include DD/ID, ranging from mild to severe, and nonspecific facial dysmorphisms. No clear phenotypic differences were observed between patients with an intragenic *STAG1* deletion and those with a pathogenic nucleotide variant. It was concluded that *STAG1* is a new cohesinopathy gene that acts via a loss-of-function mechanism, but patients with *STAG1* haploinsufficiency are difficult to recognize due to the lack of a distinctive clinical phenotype.

PWG: ANIMAL, PLANT, AND COMPARATIVE CYTOGENETICS.

Co-ordinators:
J.S. (Pat) Heslop-Harrison (UK),
Valérie Fillon (F)

Ten abstracts were selected for a short oral presentation during the workshop. The studies cover various aspects of animal and plant cytogenomics.

Dimitij Dedukh (Saint Petersburg, Russia) discussed genome elimination before meiosis in diploid and triploid hybrids of water frogs. Indeed, looking at gonads of parental species and hybrid tadpoles, many micronuclei were seen but not associated with cell death (as no signals in germ cells with micronuclei were seen when using caspase3 immunolabelling). Genome elimination was gradual over several cell cycles. He suggested mechanisms of genome elimination: budding from interphases, lagging at mitosis, or a combination of both events.

Martina Flegrova (Budejovice, Czech Republic) talked about sex chromosomes of looper butterflies, screening for sex chromatin presence or absence. Extending her analysis by comparative genomic hybridization (CGH), she and her co-authors concluded that sex chromatin was not a reliable marker of W chromosome presence but can be used as an indicator of W chromosome rearrangements. She is now looking at the reproductive consequences of these changes in loopers and the sex determining genes.

Maria Kyulak (Saint Petersburg, Russia) described new tandem repeats in Japanese quail (*Coturnix japonica*), a poultry species considered a model in development, behavioural, vertebrate physiology and disease studies. 23 tandem repeats (representing at least 4.8% of the Japanese quail genome) were found. FISH with specific oligonucleotide probes revealed that

some of them contribute to the heterochromatin of the short arms of CJA3 and CJAa, and microchromosomes. In addition, repeat CjapSAT was found in pericentromeric heterochromatin of CJA 1-6 and 3 pairs of michrochromosomes, as well as in p- and q-arms of CJAW.

Francesca Dumas (Palermo, Italy), by using both classical and molecular cytogenetics, described the genomic organization of repetitive DNA in *Graphiurus platyops* and *G. ocularis* genomes. Karyotype reconstruction with G- and C-banding showed the same diploid number (2n=46), with only bi-armed autosomal chromosomes in *G. ocularis* but 5 acrocentric pairs in *G. platyops*. FISH of telomeric (TTTAGG)n probes in both species revealed signals at the centromeres of all bi-armed chromosomes and at the terminal positions, suggesting that their dispersion could be linked to different mechanisms, such as chromosomal rearrangements and telomeric sequence amplification). There are another 15 species in the genus, so the evolutionary order of changes and consequences for speciation may soon be elucidated.

Ioana Nicolae (Balotesti, Romania), reported on data from the last 5 years from cytogenetic investigation of both cattle and river buffaloes (all females) with reproductive disturbances. From 209 investigated animals (144 cattle and 65 buffaloes), 31 of them (22 cattle and 9 buffaloes) showed chromosome instability by using a sister chromatid exchange SCE-test. Also a case of 2n=49,X in a sterile buffalo female with prominent withers and tight pelvis was found. There was some suggesting environmental pollutants may account for the high levels of SCE detected.

Also from Romania, **Dana Pusta** (Cluj-Napoca, Romania) presented an overview of the most frequent chromosomal anomalies in domestic animals and their consequences oN the reproductive traits. Particular attention was reserved to the disorders of sexual development (DSD) in cats, dogs, horses and cattle.

Alsu Saifitdinova (Saint Petersburg, Russia) discussed the NOR-transposition in the genome of Japanese quail which shows three pairs of active NOR chromosomes, while vast majority of bird species have a single pair of NOR-chromosome. Using primers to the conserved region of the 18S ribosomal RNA gene, rDNA was amplified from the quail genome karyotype and fragments of them were localized by FISH on short heterochromatic arms of all acrocentric chromosomes in the complement. In addition, a set of the fragments was cloned, sequenced and analyzed bioinformatically and revealed chimeric sequences containing fragments of transposable elements, fragments of MHC genes and some others. Lampbrush chromosomes preparations were particularly valuable to show transposable elements containing rDNA derivatives that formed lateral loops. Following the seminal work on lampbrush chromosomes of the late Professor Herbert Macgregor (1933-2018), Alsu will now continue running the Lampbrush Chromosome website, now at http://spass-sci.ru/lbc.

Mariano Rocchi (Bari, Italy), the President of ECA, was pleased to be able to present work with Doron Tolomeo, who could not be present. He showed data on Cercopithecini monkeys, the most karyotypically diverse tribe of Old World monkeys where 2n varies from 48 to 72. Detailed BAC-FISH assays were used to study the chromosomes of four species: *Chlorocebus ethiops* (CAE), *Erythrocebus patas* (EPA), *Cercopithecus mitis albogularis* (CAL) and *Cercopithecus petaurista* (CPE). Starting from an ancestral form, four variant forms were traced after a series of common inversions as a selective advantage. Although heterozygous inversions should produce unbalanced gametes, crossing over may be suppressed. This may be due to multiple rearrangements that occurred in the centromeric regions of these chromosomes or the presence of an evolutionary new centromere in the last common ancestor of CAL and CPE.

Anna Zlotina (Saint Petersburg, Russia) refers to chromomere organization and genomic context, by using microdissection on chicken giant lamp-brush chromosomes (LBCs) LBCs. Subsequent FISH on LBCs allowed to map the microdissected regions precisely and to evaluate

their transcriptional activity in growing oocytes. The data on genomic context of individual chromomeres were obtained by high-throughput sequencing providing information on chromomeres' size and genomic boarders indicating that prominent marker chromomeres are about 4-5 Mb in size, while common chromomeres - 1.5-3.5 Mb. Analysis of genomic features showed that the majority of chromomeres combine gene-dense and gene-poor regions, while massive loopless DAPI-positive chromomeres lack genes and are remarkably enriched with different repetitive elements.

Stefan Mueller (Munich, Germany) reported new data on the Western mosquitofish (*Gambusia affinis*), a species collected from lakes at Mondsee, near the ECC Congress site in Salzburg. In particular, the genomic region from the *G. affinis* amt gene localized on the long arm of the W chromosome (Wq) by FISH was dissected using exonic PCR probes. By using intra- and interspecific CGH and comparative expressed sequence hybridization (CESH), as well as FISH with rDNA and oligonucleotide repeat probes, and immuno-fluorescence, it was possible to demonstrate that the long arm of the *G. affinis* W chromosome is enriched for repetitive sequences. The study suggests that certain expressed noncoding elements from the amt genomic region with architectural localization along Wq may play a role in sex specific gene dosis compensation in this ZZ/ZW system.

Overall, the Animal, Plant and Comparative Cytogenetics working group gave an exciting opportunity to hear about a diverse range of topics showing the value of cytogenomic work in evolutionary and developmental terms, with many implications for disease and speciation. The discussions started during the well-attended session continued through the conference and particularly during the main sessions during the Congress.

Participants of the PWG meeting at the 12th ECC