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E.C.A. on Facebook

As mentioned in the previous Newsletter, E.C.A. is on Facebook.
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/**

You can find a selection of interesting Facebook posts in this Newsletter starting at page 18.
Please contact us (mariano.rocchi@uniba.it) if you wish to share an interesting news item or a pertinent article.

ISCN 2020 compared to ISCN 2016

Konstantin Miller and Kamlesh Madan

ISCN 2020 has recently appeared (J. McGowan-Jordan, R.J. Hastings, S. Moore (Eds.): An International System of Human Cytogenomic Nomenclature (2020), Cytogenet Genome Res 2020; 160:341-503, S. Karger AG, Basel).

We have made a detailed comparison between the ISCN 2020 and the previous version. The major changes are in the chapters on Microarrays and Sequence based Nomenclature which have become much longer with many more examples. These changes reflect the rapid developments and changes in nomenclature in these fields in the last four years. In the sections on classical cytogentics the text has been made considerably easier to read by either dividing sections into subsections or by presenting data using bullet points instead of large blocks of text. Although the format of the book is the same as the 2016 version, there are 24 more pages in the 2020 edition. The extra information has resulted in changes in pagination.

Most of the changes and/or additions are indicated by a grey bar in the right margin; these are shown below in green. There are, however, many changes and additions (for example in chapter 9) that may be as important and relevant as the ones with the gray bars that have not been indicated; these are given in red below. There are other changes such as those in the examples of karyotypes or new examples following the new rules; these are given in blue. The items that have been deleted (with respect to 2016) are not indicated in ISCN 2020 but are noted below. Our comments are indicated in pink

Here are the details of the changes in ISCN 2020 as compared to ISCN 2016:

Contents: In the ISCN 2020 there is double page numbering; the first refers to the journal article in Cytogenetics and Genome Research and the second to page numbers for ISCN booklet as in the past.

At the top of the contents page the readers are asked to use the page numbers of the journal article when citing ISCN 2020. As in 2016, the reference is given at the bottom of each page: Cytogenet Genome Res 2020;160:341-503.

Chapter 1 Historical introduction:

Page 6: A new section1.7, 2017-2020 has been written by Jean McGowan-Jordan and Ros Hastings.

Chapter 2 Normal Chromosomes:

Page 7 section 2.1. line 4:

- Added: Gothenburg 2019

Page 8, end of section 2.2.1

- added 'The definition of metacentric … 1969).'

Chapter 3 Symbols, Abbreviated Terms, and General Principles

Pages 34, 35 & 36

- Deleted symbols and abbreviations:
  - Approximate sign (~) (but replaced by tilde (~) see below)
  - cgh Comparative genome hybridization
  - curly braces ({}) Indicate differences …
  - end Endoreduplication

- New symbols and terms added:
  - cha Chromoanasynthesis
  - delins Sequence change with nucleotides of…
  - dinh Derived from chromosome abnormality of parental origin (Useful where disclosure of parent's karyotype is illegal)
  - dmat Derived from chromosome abnormality of maternal origin
  - dpat Derived from chromosome abnormality of paternal origin
  - g. Genome with reference to the genomic sequence (was missing in 2016 list but was already used)
  - greater than (>) Greater than
  - hyphen (-) Hyphen … chromosome band at low resolution
  - ins Insertion; insertion of nucleotides (description extended)
  - inv Inversion; inverted in orientation relative … sequence (description extended)
  - rev Reverse (words deleted: including comparative genomic)
  - sseq Shallow next-generation sequencing
  - sup Additional (supernumerary)sequence not attached …
- New but not marked
  - tilde (~) Denotes intervals … (replaces approximately, see above)

Page 37

- A New table 'General Principles' concerning order of presentation of findings for various methods has been added.

Chapter 4 Karyotype Designation

4.1 General Principles

Page 38

- Line 7. 45, X? Where the sex is not disclosed (Useful where disclosure of fetal sex is illegal)

Page 39

- Point 6: tilde replaces approximate sign
- Point 8: added: '… in each cell line or…'
- Point 9:
  - [10] added as number of cells for each of the 2 cell lines in both examples (according to the rules for ranking in the karyotype in point 11 & 12)
  - The word abbreviations replaces triplets
- Points 11&12: cell lines replaces clones and the number of cells are indicated [ ] for all cell lines

Page 40

- Last point on Endoreduplication:
  'Technologies such as … number change' replaces 'An endoreduplicated … end 46,XX' (Useful suggestion to distinguish between Endoreduplicaion and other mechanisms)-

Page 41

- Point 1 line 6: sentence added: 'However … complement in the parent' (following the general principle of the use of dmat, dpat and dinh)
- line 6 from below: sentence added '…; however, if an overall … in accordance with national regulatory agencies as applicable.' (indicates that national rules concerning specification of breakpoints should be followed here)

Page 42

- 4.3.1.1 Two-break Rearrangements. Text of lines 1, 2, 4 & 5 from 2016 concerning inversions is

combined and changed; the key point is: Whether the two breaks are in one arm or different arms of the single chromosome, the breakpoints are specified from pter to qter. (This new rule has been applied throughout the ISCN 2020; the breakpoints in karyotypes in chapters 8, 9 etc., have been changed accordingly)

- Breakpoints adapted in example
  - 46,XX,inv(2)(p23p13)

Page 43

- Phrase '…i.e., the breakpoints of the inserted segment are specified with the one closer to the pter of the recipient chromosome listed first' replaces the one in 2016. (Rule for breakpoint specification for insertions)
  Breakpoints and text in the two examples have been adapted accordingly
  - Line 9: Text for ins(2)(q13p13p23) is changed
  - Line 11: Text for ins(2) (q13p23p13) is changed

Page 45

- Line 3 from below: added '(i.e., breakpoints not included)' (indicates how a karyotype can be abbreviated when written more than once)

Pages 46 & 47

- Lines 4 from below on both pages: 'dmat' replaces 'mat' of 2016

Chapter 5 Uncertainty in Chromosome or Band Designation

Page 49

- Second example from the top is new
  - '46,XY,der(5)ins(5;?)(q42;q13) A derivative… q32'
- Last sentence of the page is new 'Further testing is required to differentiate between the two possibilities'

Chapter 6 Order of chromosome Abnormalities in the Karyotype

No changes

Chapter 7 Normal Variable Chromosome Features

Page 53

- 7.1 In the introduction to the section 5 extra lines have been added 'The following sections ….transplant' (It is for the first time that ISCN strongly recommends that variants should not be included in the ISCN nomenclature)

Chapter 8 Numerical Chromosome Abnormalities

Page 56

- 8.2 Two extra examples (7 and 8 from the top) added:
  - mos 45,X[25]/47,XXX[12]/46,XX[13] A mosaic... was found in 13 cells'
  - mos 47,XXX[25]/45,X[12]/46,XX[13] A mosaic... was found in 13 cells'

Page 58

- 8.4 The whole section 8.4 on Uniparental Disomy has been deleted.

Chapter 9 Structural Chromosome Rearrangements

Whereas some changes in the text on Duplications (9.2.5) and Inversions (9.2.10) have been marked with a grey bar, other changes, which are equally important/relevant, such as texts on Dicentrics (9.2.4), Isochromosomes (9.2.11), Telomere Associations (9.2.16), Reciprocal translocations (9.2.17.1) and Robertsonian translocations (9.2.17.3), have not been marked. These changes are

given in red.

Page 61

- 9.2.2 Two new karyotypes & text (6th form bottom & the last) added:
  - '46,XX,del(4)(p15.2) … entire long arm.'
  - '46,XY,del(20)(q11.2-13.1q13.3)…and 20q13.3'
  - breakpoints & text changed 46,Y,del(X)(p21p11.4) (new pter to qter rule)

Page 62

- 9.2.3 Derivative Chromosomes
  - Extra phrase added in line 6: '…, nor those resulting from malsegregation.'
  - Terms dmat, dpat and dinh used in karyotypes (in 2016: mat, pat, inh)
  - Change in the short form of the karyotype in second example from below: 46,XY,der(9)inv(9)(p23p13)del(9)(q22q23) (was p13p23 in 2016) (new pter to qter rule)

Page 63

- 9.2.3 (continued): Extra examples added (5th and 6th from the top)
  - '47,XX,+7,der(7)t(1;7)(q12;p22)x2 In …chromosome1'
  - '47,XY,+der(4)t(4;11)(q21;q23),t(4;11)(q21;q23) In … translocation'

Page 64

- 9.2.3 (continued):
  - Karyotype and text changed (5th example from above, last one on p62 in 2016) '47,XY,+der(8)r(1;8;17) …' (Note: the word *mos* is deleted and in text supernumerary ring replace *mosaic ring*)
  - Karyotype order of breakpoints and text changed (6th from above, 1st on p 63 in 2016): '46,XX,der(1)del(1)(p34p22)ins(1;17) ... of insertion'
  - Extra karyotype and text (2nd from the bottom) added: '47,XX,t(9;22;6)(q34;q11.2;p21),+der(22)t(9;22;6) ... three-way translocation.'

Page 65

- 9.2.3 (continued):
  - An extra phrase added in text of karyotype (2nd from the top) 46,XY,ider(9) etc. '… at band 9p13 with band 12q22 closer to 9pter than band 12q13.'
  - Extra karyotype and text added (last one on the page): '46,XY,der(9)t(9;22) … generated by a t(9;22)'

Page 67

- 9.2.4 Dicentric Chromosomes
  Text of the first paragraph of 9.2.4 divided into 3 (was one in 2016) with changes and new recommendations
  (Note: *dic* and *idic* have been separated in 2020)

Page 69

- 9.2.5 Duplications - text changed:
  '… by the order of bands from pter to qter' (in contrast to 2016 '… with respect to the centromere') (Also here, as for inversions 4.3.1.1 and insertions 4.3.12 above, many karyotypes and texts have been adapted below.)
- Two new karyotypes and text added
  - '46,XX,dup(1)(p34p31) … in orientation'
  - '46,XX,dup(1)(p31p34) … duplicated segment'
  - Phrase added in the text of the last karyotype in section 9.2.5: '… and 1q25, in reversed orientation relative to pter and qter.'

Page 70

- 9.2.8 Homogeneously Staining Regions
  - Extra karyotype and text added (2nd example): '46,XY,hsr(21)(q22) … in band 21q22'

Page 71

- 9.2.9 Insertions:
  Band order in the short version and text changed in the three karyotypes while the long versions remain as in 2016. (new pter to qter rule)
  - '46,XX,ins(2)(p13q31q21) … band 2q21.'
  - '46,XX,ins(2)(p13q21q31) … band 2q31.'
  - '46,XX,ins(5;2)(p14;q32q22) … specified first.'

Page 72

- 9.2.9 (continued)
  Band order in the short form and/or text changed in 4 karyotypes (to adapt to new rule of pter to qter) :
  - 1st karyotype: order of bands and text
  - 2nd karyotype: only the text
  - 3rd karyotype: only the text
  - 5th karyotype: order of bands and text
- 9.2.10 Inversions:
  - Extra text added: 'In all cases … specified first'
  - Band order changed in first karyotype and sentence from 2016 'The breakpoint … first' deleted

Page 73

- 9.2.11 Isochromosomes:
  Text (starts on page 72) extended by 4 lines 'The isochromosome designation is … section 9.2.3' (gives the formation and description of iso chromosomes and refers to 9.2.3 for complex isochromosomes)
  - Extra sentence added to text of 2nd example:
    'This is unbalanced as there is a single copy of Xp and 3 copies of Xq'
  - Extra karyotype and text, 5th example from top added:
    '45,XX,-21,i(21)(10) … chromosome 21'
  - The last sentence in section 9.2.11 has been deleted in the 2020 version.

Page 74

- 9.2.12 Marker Chromosomes (continued from page 73)
  - Last karyotype: the number of double minutes has been changed from 9~34 to 9~50

Page 76

- 9.2.15 Ring Chromosomes (continued from page 75).
  There are two examples, the 1st with a large deletion and the 2nd with no significant deletion.
  - 46,XX,r(7)(p15q31) (the breakpoints of the single example of a ring 1n 2016 have been changed in 2020 from p22q36 to make it an example with a significant deletion)
  - 46,XX,r(20)(p13q13.3) new karyotype and text (with very distal break points and practically no deletion)

Page 77

- 9.2.16 Telomeric Associations:
  Text is added to say that this is typically a single cell abnormality and that true chromosome fusion

with terminal breakpoints is not proven.

Page 78

- 9.2.17.1 Reciprocal Translocations:
  The text is changed and extended. It defines a reciprocal exchange and explains a balanced translocation.

Page 81

- 9.2.17.3 Robertsonian Translocations:
  The text is changed and extended. It states that der is preferred to rob. A sentence about acquired abnormalities has been moved up (was at the end in 2016)
    - The 2nd option of a karyotype with rob has been deleted from the last two karyotypes on the page.

Page 82

- 9.2.17.3 Robertsonian Translocations:
    - The 2nd option of a karyotype with rob has been deleted from the first two karyotypes on the page. The text of the 1st is reduced but is essentially the same as in 2016

Chapter 10 Chromosome Breakage

Page 87

- 10.2.1 Non-banded Preparations
    - Line 1 on page 87: '(commonly alternating disomy and heterozygous loss)' replaces '(normal, gain or loss)' from 2016.

Chapter 11 Neoplasia

Page 89

- 11.1.1 (continues from page 88)
    - Line 8 from above: '… but, if appropriate, can be discussed in the interpretation' replaces the 2016 '…but should be discussed in the interpretation' (This refers to additional abnormalities seen in a single cell but not proven to be present with another method. So, according to ISCN 2020, they don't have to be mentioned in the report; it is left to your discretion).
- 11.1.2 Clone Size
    - Extra karyotype and text added (3rd karyotype) '46,XX,t(9;22)(q34;q11.2)[18]/45 ...chromosome 7.'
    - Bands of t(8;21)not repeated in the second cell line

Page 90

- 11.1.4 Stemline, Sideline and Clonal Evotution
    - End of 2nd paragraph: 'In such instances idem is preferred' is emphasized in BOLD letters in the 2020 version.

Page 91

- 11.1.4 (continued)
    - In 3 karyotypes (5th, 6th, and 7th example from the top) '+17,+20' replaces '….' of 2016

Page 92

- 11.1.4 (continued)
    - In the 1st example '+1,+12,+14' has been added and '+15,+16,+18,+20' replaces '….'
    - In the 2nd example the chromosome number of first cell line changed from 53 to 45 and in the second cell line from 57 to 49
    - In 3rd example the chromosome number of first cell line has been changed from 49 to 47

and in the second cell line from 52 to 50.
'+12' replaces '….', the text is changed and expanded.

- 11.1.5 Composite Karyotype
  - 3 lines added to text: '…;in an otherwise normal … nonclonal abnormalities' (random losses and gains should not be included in the nomenclature).

Page 94

- 11.1.6 Unrelated clones:
  - 2nd karyotype '+5,+5,+8,+11' replaces '….'
  - Table on ploidy levels has been moved from 11.1.6 to 11.2

Page 95

- 11.2 Modal number
  - New sentence 'Ploidy levels are recommended but exceptions may be made if biologically significant'
  - Extra karyotype and text added): '81<3n>,XXX, … near tetraploid range.'
- 11.3 Constitutional Karyotype
  - Extra karyotype and text added: '47,XXYc[5] … X chromosome'

Page 96

- 11.3 (continued)
  - 7th karyotype: Extra cell line 46,XX[8] and text 'The normal … listed last'
  - In paragraph in middle 'To describe acquired…' replaces 'To appropriately express acquired …'

Page 97

- 11.4 Counting Chromosome Aberrations
  - Whole new section with a table has been added: 11.4 Counting Chromosome Aberrations

Chapter 12 Meiotic Chromosomes

No changes

Chapter 13 *In situ* Hybridization

Page 106

- 13.1 Introduction: The last 4 lines from 2016 'If FISH further … banded karyotype' replaced by 2 paragraphs in 2020:
  - 'By convention, … (e.g., BCR/ABL1).' (information on the use of hyphen (-) versus slashes (/)
  - 'Where multiple … lines without periods'(information on the karyotype order in case of multiple techniques)

Page 107

- 13.2 Prophase/Metaphase in situ Hybridization (ish)

This section is much better organized and easier to read in the 2020 version because the information is presented using bullet points instead of everything one after the other in long paragraphs.

  - The first point is new but it is the same as the one that was removed from 13.1 of 2016 (see above): 'If FISH further … banded karyotype'
  - Point 8: a phrase added: '… or a single designation … in the report'

- Point 10: added 'When FISH results are … ish descrition'
- Point 12: This is an extra new point 'The breakpoints … nomenclature'

Pages 107 to 114

- Rest of Section 13:2

  In ISCN 2016 there were 58 examples (pages101 to 106). Most of these were directly under 13.2 and there were two further subdivisions 13.2.1 and 13.2.2.

  In ISCN 2020 Section 13.2 has 70 examples classified under 7 subdivisions 13.2.1 to 13.2.7 (the last two 13.2.6 and 13.2.7 are the same as 13.2.1 & 13.2.2 of 2016 and are unaltered). Some of the 70 examples are new, some are the same as 2016 and some have been altered. It is not useful or relevant to compare individual karyotypes with the 2016. The whole section 13.2 should be considered as new with a new classification:

  13.2.1 Normal Signal Pattern
  13.2.2 Abnormal Signal Patterns with Single Probes
  13.2.3 Abnormal Signal Patterns with Multiple Probes
  13.2.4 Abnormal Mosaic and Chimeric Signal Patterns with Single or Multiple Probes
  13.2.5 Oncology-Specific Exceptions where Multiple Copies of the Same Gene are present
  13.2.6 Use of dim and enh in Metaphase in situ hybridization
  13.2.7 Subtelomeric Metaphase in situ Hybridization

Page 115

- 13.3 Interphase/nuclear in situ Hybridization (nuc ish)
  - At the end of 13.3, 'When contig probes …in the report' replaces 'If a collection …slant lines'
- 13.3.1
  - Added in line 4: 'For simplicity and readability, the short form is preferred'
  - Point 4: phrase added 'If the study is on a cancer specimen, the number of cells scored is placed in square brackets for each technique' (significant change)
  - Point 5: is new 'Cell lines and clones are listed from largest to smallest number of cells'
  - Point 6: Extra 2 lines added 'Normal results from multiple hybridizations can be combined in a single set of parentheses; however, if different number of cells are studied in multiple hybridizations in a cancer specimen, the results are presented in separate sets of parentheses'
  - Point 7 is new 'If two or more techniques … each is reported within the string, separated by a period (.)'
  - New 3 lines added at the end of 13.3.1: 'Caveats of techniques … not presented in the nomenclature; instead they should be stated in the interpretive text' (significant new point)

Pages 116 to 118

The examples (some changed) in the rest of section 13.3 have been classified into 3 new sections. As for 13.2 above, it is not useful or relevant to compare individual karyotypes. The new sections are:

13.3.2 Normal Interphase Signal patterns
13.3.3 Abnormal Interphase Signal Patterns
13.3.4 Donor versus Recipient

Page 118

- 13.3.5 (was 13.3.2 in 2016)
  - New sentence: 'For simplicity and comprehension the short form is preferred'

Page 119

- 13.3.5 (continued)

- In the examples on this page the number of cells has been changed from [400] to [100]
- Phrase added to top example '… describing the relative position of the signals to one another using **sep** and **con**'
- 3rd example on the page: words 'an unusual' replace 'a strange'

Page 120

- 13.3.5 (continued)
  - 3rd example: [200] added at the end
  - 4th example: '… as follows using amp' added at the end
  - 4th example also [180] added at the end
  - 5th example: '…FOXO1 amp)[100]' replaces 'FOXO1)amp'

Page 121

- 13.3.5 (continued)
  - 13.3.5.1: [100] replaces [400]
  - 13.3.5.2: 1st example [103/200] replaces [300/400]; 2nd [110/200] replaces [300/400]
  - 13.3.5.3: 1st example [200] replaces [400]; 3rd example [100/200] added
  - 13.3.5.4: refers to break-apart probes: additional sentence 'The long form conveys both the normal situation followed by the signal fusion pattern seen'
  - 13.3.5.4: 1st example [200] replaces [400]; 2nd example [200] replaces [400]

Page 122

- 13.3.5 (continued)
  - 1st example: [213] replaces [200]; added to text '… or because … derivative chromosome'
  - Order of 3 examples is altered. In 3rd one [198] replaces [200]
- 13.3.5.5 Whole new section Tricolor Probes with 5 examples has been added

Page 124

Chromosome Comparative Hybridization (cgh) which was 13.6 in 2016 has now been integrated into chapter 14 on Microarrays (see aCGH in introduction to chapter 14); the abbreviation cgh has been deleted (see also changes in chapter 3 above).

In the 2020 version 13.6 Multi-color Chromosome painting and 13.7 Partial chromosome paints have not been changed and are the same as 13.7 and 13.8 of 2016.

Chapter 14 Microarrays

This Chapter is totally changed, and should be considered as new.

It is nearly twice as long and is divided and subdivided into 14 headings (as compared to 4 in 2016) including some news ones such as Polar bodies. There are many more examples and the way of writing them is new. It is therefore not useful or relevant to make any detailed comparisons with the 2016 version.

Most of the basic changes are indicated in the margin. The changes worth mentioning are:

Page 125

- Line 6 from below: 'In the short form the nucleotide numbers are given either with or without commas to indicate thousands and millions" (no change for the detailed form)
- Last two lines: 'It is acceptable to use a mixture of detailed form and short form…'

Page 126

- First paragraph is about retrospective changes in banded karyotypes following microarray analysis
- Second paragraph concerns for highly complex karyotypes.
- 14.2.2 line 2: '… the aberrations of sex chromosomes are listed first followed by the

autosomes…' (these were 'last' in 2016)

Page 136

- 14.2.7 in the middle of the page: A revised definition of chromothripis (**cth**)
- Line 6 from below: term Chromoanasynthesis (**cha**) is new

Page 137

- 14.2.8 is a new section on Polar bodies
- Paragraph 4 of 14.2.8: Term **cht** (chromatid) used to describe polar body results
- Paragraph 6 of 14.2.8: Term "**sseq**" used for shallow NGS and replaces "**arr**"

Chapter 15 Region Specific Assays

This Chapter is totally changed, and should be considered as new.
It is twice as long. There are many more examples and the way of writing them is new. It is therefore not useful or relevant to make any detailed comparisons with the 2016 version.
The basic changes are indicated in the margin. Some worth mentioning are:

Page 140

- 2nd paragraph in 15.1: When a kit is used, the kit name must be given.
- Last paragraph in 15.1: sex chromosomes should be listed first (as in Chapter 14)

Pages 140 -143

Section 15.2 has been split into:

- 15.2 Examples of RSA Nomenclature for Normal and Aneuploidy
- 15.3 Examples of RSA Nomenclature for Partial Gain or loss
- 15.4 Examples of RSA Nomenclature for Balanced Translocations or Fusion genes (same as 15.3 in 2016)

Chapter 16 Sequence–Based Nomenclature for Description of Chromosome Rearrangements

This Chapter is totally changed and should be considered as new:

- Introduction has some editorial changes
- General Principles have been extensively revised
- The subdivisions are more or less the same; there is an extra section 16.4.
- The examples and the way of writing them is new

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Meeting Report 7th Asian Pacific Chromosome Colloquium (APCC7)

The **7th Asian Pacific Chromosome Colloquium (APCC7)** was held during November 26-27, 2020, in Bexco, Busan, Korea. The Asian Chromosome Colloquium (ACC) was established by a few chromosome scientists to exchange research materials and ideas for chromosome research during the 18th International Congress of Genetics in Beijing in 1998. Then, the first ACC was held in 2000 in Bejing and the ACC was extended to Asian Pacific Chromosome Colloquium (APCC) at APCC6 which was held in Canberra, Australia, in 2018.

The APCC7 was held in parallel with the **International Congress of Genetics Society of Korea 2020 (ICGSK_2020)**. The theme was "***Chromosome sciences in genomics era***". Because COVID-19 pandemic was prevalent globally, the conference was held by virtual online presentation. There were one plenary lecture and five symposia sessions. All lectures were presented by pre-recorded files or ZOOM presentation. Q/A were done by ZOOM communication. Sixty-seven e-posters were posted.

Plenary lecture was given by **Professor Pat Heslop-Harrison at University of Leicester, UK** with a title "The Genome Landscape: Consequences of repetitive DNA organization and evolution in diploid and polyploid species". Various kinds of repetitive DNAs are major residents in eukaryotic genome. He presented long-molecule sequencing technique in genome assemblies and chromosome studies in building the complete picture of repetitive DNA organization and evolution. He demonstrated the usefulness of these approaches in grass, Musaceae and Brassciaceae species for chromosome studies. With the results, he proposed developing models of the processes occurring during repetitive DNA evolution in terms of diversification and homogenization, which can be used for chromosomal and evolutionary markers.

APCC7-I was the session on **Chromosome Structure** in which four topics were presented. The first speaker was **Professor Fangpu Han at Chinese Academy and Science, Beijing, China** with a title "Genetics and epigenetics of plant centromere". With maize B-A translocation chromosomes, he demonstrated that epigenetics of plant centromeres was involved in inactivation and nondisjunction of chromosomes during meiosis. He also showed their recent data on the molecular mechanisms for centromere formation and maintenance. The second speaker was **Professor Sang-Hee Shim at Korea University, Korea** with a title "Super-resolution fluorescence imaging of chromatic structures in living cells". Spatial organization of genome is crucial in regulation of gene expression. A switchable protein was able be captured by reversible fluorescence recovery. She showed a movie that was composed of hundreds of super-resolution frames in which various sizes and shapes of a genomic loci in a cell reflect different states of the cell or local nuclear environment. The third speaker was **Professor Kichii Fukui at Osaka University, Japan**, with a title "Focused ion beam/SEM method reveals the inside structure of a chromosome". In his talk, the fusion of nano-technology in microscopy was demonstrated how useful in studies of functional and structural studies of chromosomes. He overviewed the recent advances of nano-technology in chromosome research. Moreover, he also summarized the advantages and disadvantages of these technologies based on the achievements that were already attained. The last speaker of this session was **Professor Guohong Li at the Chinese Academy of Science, Beijing, China** with a title "Dynamic regulation of higher-order chromatin structures in gene regulation and epigenetic inheritance". Eukaryotic chromosomes are highly packed structures and the dynamics of chromatin structure plays a critical role in transcriptional regulation and other DNA related biological processes. Using cryo-electron microscopy, he demonstrated the repeating tetra-nucleosomal structural units and the roles of asymmetries and polarities of nucleosome in folding the chromatin fibers.

APCC7-II was a session on **Sex chromosomes and B-chromosomes**. Four speakers presented their results. The first speaker was **Professor Nam-Soo Kim at the Kangwon National University, Korea**. His topic was "Distribution of B-chromosome aneuploids and genetic diversity of the Korean accessions of *Lilium amabile*". *L. amabile* is an endemic Korean lily that are distributed throughout the Korea. In his talk, the B-chromosome diversity, geographic distribution and retrotransposon diversity were not related among the accessions of Korean lilies. The second speaker was **Professor Cesar Martin at Sao Paulo University, Brazil** with title "B chromosomes in cichlid fish: from cytogenetics to functional genomics". Cichlid fishes are good model in studies of B-chromosome. He showed that the B-chromosomes in cichlid are enriched with genes, relics of genes, transposable elements. One of the most significant findings by his group was that most enigmatic characteristics of Bs in cichlids is their genic contents that are related to cell cycle and chromosome structure, and their influence over sex rates. He speculated that the relation with cell cycle and sex might be related with the drive of Bs during cell divisions. The third speaker was **Professor Andreas Houben at the Leibniz Institute of Plant Genetics and Crop Research (IPK), Germany** with the title "Drive and elimination of B chromosomes – different sites of the same coin?". Chromosome elimination is often found in interspecific crosses in plants. He presented detailed analyses of programmed B-chromosome elimination using goatgrass *Aegilops speltoides* as a model. He showed

several lines of evidence that elimination of B chromosomes is s strictly controlled and highly efficient root-specific process in a hybrid plants between *Aegilops* and *Secale*. Non-disjunction of chromatids and anaphase lagging of B-chromosomes lead to micronucleation which is degraded to lead degradation of B-chromosomes. As well, spindle asymmetry also leads to the B-chromosome drive in pollen mitosis. Thus, he proposed that the type of spindle organization (symmetry vs asymmetry) determines whether drive or elimination of B chromosomes becomes the consequences. The last speaker of the session was **Professor Ikuo Miura at Hiroshima University, Japan** with the title "Optimistic destiny of sex chromosome evolution in frogs". Sex chromosomes can be homomorphic (XX or ZZ) or heteromorphic (XY or ZW). Because meiotic recombination is restricted between X and Y or Z and W chromosomes, the Y or W chromosomes suffer decaying genetic materials, shrinking, and eventually be gone. In amphibians, sex chromosomes are still homomorphic in both sexes in around 96%. Professor Miura demonstrated evolutionary mechanisms to rejuvenate the heteromorphic sex chromosomes by recycling and interpopulation hybridization in Japanese frog, thus to stay or return back to homomorphy of sex chromosomes.

APCC7-III was a session of **Chromosomes and Evolution** with five speakers. The first speaker was **Professor Jin Hoe Huh at Seoul National University, Korea** with a title "Epigenetic regulation of transposable elements and hybrid genome stability in x*Brassicoraphanus*". *Brassica rapa* and *Raphanus sativus* belong to the Brassicaeae, but genomes of both species are highly divergent with unique TE compositions. He showed that the DNA methylome profiles are similar in both species, but certain classes of TEs were differently methylated. However, the hybrid between them x*Brassicoraphanus* revealed similar methylation levels, from which he proposed that interactions between two subgenomes were likely determine the epigenetic landscape in the hybrid. The second speaker was **Professor Dal-Hoe Koo at the Kansas State University, USA** with a topic "Extrachromosomal circular (eccDNA) drives glycophosate resistance in weeds". EccDNA ranges in size from a few hundred base pairs to megabases and is one of the characteristic features of genome plasticity in eukaryotes. The

eccDNA can accumulate its copies in response to stress or ageing. Professor Koo reported that eccDNA-mediated amplification of *EPSPS* gene, the molecular target of herbicide glyco-phosate, in *Amaranthus palmeri* which is a highly problematic weed. Next speaker was Professor **Yasuhiro Mukai at Osaka Kyoiku University, Japan** with "Asian luminaries in plant chromosome research: contribution to polyploidy and chromosome evolution". It is always good to show our respect to seniors and give them credits what they did for pioneering research. Professor Mukai reviewed Asian pioneer chromosome scientists during the past 100 years with a phrase "*Studying the past to learn new things*". The term "genome" was first coined by a German botanist Hans Winkler to mean "a set of chromosomes in a gamete" in 1920, but a Japanese plant geneticist Hitoshi Kihara defined further it as "a set of the minimum chromosomes essential for the survival of living organisms" from his research in wheat. In 1918, Tetsu Sakamura, a mentor of Kihara, discovered three ploidies of 2x, 4x, and 6x in wheat. Professor Mukai also introduced other key cytogeneticists including Jang-Chun Woo for his research in brassica ploidy known as "U triangle", Arun Sharma and Archana Sharma for their contribution of chromosome studies in angiosperms and classical cyto-genetics book "*Chromosome technique: theory and practice*". The contribution of Gurudev Khush from India worked at IRRI in rice genetics and cytogenetics was also credited. Bikram Gill from India working at Kansas State University, USA and Rudy Appels in Australia were credited for their contribution in modern molecular cytogenetics. Next speaker was **Dr. Jungnam Cho at CAS-JIC Centre of Excellence for Plant and Microbial Science, Shanghai, China.** His topic was "How plants recognize genome invaders". Transposon mobility can cause fatal mutations to host so that host equipped counteracting machineries including small interfering (si) RNA to silence the transposon activity. He demonstrated nicely how plant transposon RNAs undergo frequent ribosome binding stalling by their inherently unfavorable codon sequence usage, which induce the RNA truncation and the localization to siRNA bodies to be degraded. With other persuasive results, he proposed a novel insight into the regulatory mechanisms for the recognition of invasive genetic elements to maintain genetic integrity. The fifth speaker was **Professor Hyun-Hee Kim at Sahmyook University, Korea** with a title "*In silico* mining of repetitive DNA and FISH analysis for plant genome research". As shown in the plenary lecture by Pat Heslop-Harrison, repetitive DNAs are major components in eukaryotic genomes and, thus, the characteristics, abundance, and distribution of these repetitive DNA along the chromosomes can provide highly informative information in understanding the genome structure and function. Professor Kim introduced the flowchart from NGS sequencing to mining the repetitive DNAs for using in chromosome research in the genera *Panax, Brassica*, and *Raphanus.* She showed the results with pre-labelled oligonucleotide probes for five-color FISH. The last speaker in this session was **Professor Kornsorn Srikulnath at Kasesart University, Thailand** with the title "Under omics era, satellite DNA: an epoch-making element for chromosomal rearrangements, population demography, and speciation". The main theme of his talk was also about the repeatomes. Among various repeated DNAs, he utilized lineage-specific satellite repeats in delineating the vertebrate species evolution because diverse stDNA families and subfamilies coexist in the genomes of related species. With these results, he proposed that evolutionary dynamics of stDNA can provide very good insights into genome organization, population

level, and evolution in vertebrate species.

APCC7-IV was a session on **Genomics and Chromosomes** was a session that was co-hosted by ICGSK and APCC7 so that some speakers were invited by ICGSK and others were invited by APCC7. There were five speakers. The first speaker was **Dr. Yingming Bao in China National Center for Bioinformation and Beijing Institute of Genomics at Chinese Academy of Sciences, Beijing, China**. His topic was "Resources of China National Center for Bioinformation". China is a major powerhouse that generates large amount genome data. He brought a problem in handling the large genome data due to the slow data transfer by limited international internet bandwidth, language barrier, and technical issues in communication. In order to alleviate these problems, China launched establishing several national institutes including BIG Data Centre (BIDG, http://bidg.big.ac.cn) and China National Center for Bioinformation (CNCB). He introduced several other institutes and explained how other scientists can access data repositories and other variety of data resources in support of worldwide research activities. The second speaker was Professor **Hiroo Imai at Kyoto University, Japan** with a title "Evolution of taste receptors in primates". Animals evolved the taste receptors as related to their diets in specific environments. He presented functional analysis of primate taste receptors along with behavioral analysis in monkeys to demonstrate the evolution of taste receptors as related to the food selection. The third speaker was **Jiming Jiang at the Michigan State University, USA** with a title "Oligo-FISH: a cornerstone technique in the new era of plant cytogenetics". Fluorescent in situ hybridization (FISH) has been used for nearly three decades to answer various cytogenetic questions related to the structure, mutation, and evolution in the level of individual chromosome as well as in entire genomes. Utilizing robust probes is critical in obtaining successful FISH results. He introduced the Oligo-FISH technique by utilizing probes based on synthetic oligonucleotides (oligos). The oligo-based FISH can apply any plant species with a sequenced genome. He promised that the advent of oligo-FISH can overcome the lack of robust FISH probes in many plant species and will become a cornerstone technique in the new era of plant cytogenetics. The forth speaker was **Professor Peter Choi at University Pennsylvania, USA** with a title "Role of alternative splicing in maintaining integrity of the genome". Chromosome instability is a common characteristic of cancer cells, resulting in abnormalities in genome structure and copy-number. He provided evidences that perturbation of RNA splicing factors leads to defects in mitosis, suggesting that post-transcriptional regulation play an important role in maintaining genome integrity. RBM10, an RNA-binding protein, regulates the alternate splicing of a large number of genes. RBM10-deficient cells resulted in improper exist from mitotic arrest, indicating a weakened spindle assembly checkpoint which can result in the tumorigenesis. The last speaker in the session IV was **Professor Tae-Jin Yang at Seoul National University, Korea** with a title "Dynamic evolution of *Panax* species". Ginseng, an important medicinal plant, is a species in the genus *Panax*. The genome of *P. ginseng* was 2.98 Gbp with 59,352 annotated genes. It had undergone two times whole genome duplication. He provided data that support a recent allopolyploidization. He also characterized several high-copy LTR-retrotransposons that had impacted in building the current genomes in the genus *Panax*.

The APCC7-V was on the **Epigenetics and Chromosomes**. There were four speakers in this session. The first speaker was **Professor**

Masako Tada at Toho University, Japan. Her presentation title was "DNA methylation dynamics through the cell cycle in mouse embryonic stem cells". DNA methylation is crucial at the onset of cell differentiation during post-implantation development in mice. Her group compared DNA methylation patterns between two cell types differentiated from transgenic mouse embryonic stem cells: one expressed full length DNMT1 and the other of mutant DNMT1 by deletion. The results showed that DNMT1 may be able to access DNA even in heterochromatin through protein-protein interaction in the N-terminal region of DNMT1. In euchromatin, 5mC was converted to 5hmC when TET, DNA demethylation enzyme, is expressed. Thus extensive epigenetic reprogramming allows stochastic changes in DNA modification during cell cycle and embryonic development. The second speaker was **Professor Danny Chi Yeu Leung at the Hong Kong University of Science and Technology, Hong Kong** with a title "G9a plays distinct roles in maintaining methylation, retrotransposon silencing and chromatin looping". The lysine methyltransferase G9a is responsible for depositing histone 3 lysine 9 (H3K9) methylation, which is associated with transcriptional repression. His group conducted an in-depth analysis of G9a depleted and G9a catalytic mutant embryonic stem cells (mESCs). The results showed significant genome-wide loss of DNA methylation in G9a mutant cells. Epigenomic analysis revealed that G9a regulated chromatin accessibility and histone modifications in both catalytic dependent and independent mechanisms, resulting in the dysregulation of retrotransposons and serving as non-canonical promoters and novel splice sites. Thus, he proposed that the G9a regulates the epigenome, transcriptome and nuclear organization of mESCs. The third speaker was **Professor Inkyung Jung at Korea Advanced Institute of Science and Technology (KAIST), Korea** with a title "Gene regulation in 3D chromatin structure". Chromatin 3D structure is important in gene expression regulation. Disorganized 3D chromatin structure can lead to oncogenic gene expression through exposing genes to unwanted *cis*-regulatory elements. He showed widespread and complex disorganization of higher-order chromatin structure in the genome of colon cancer patients. His group used CRISPER/Cas9 to validate *TOP2B* gene overexpression to reveal its clinical indication through engineered rearrangement of 3D chromatin structure. The last speaker in this session was **Professor Hyoungpyo Kim at Yonsei University, Korea**. The title of his talk was "The role of chromatin insulator CTCF in dendritic cells". Dendritic cells (DCs) are professional antigen-presenting cells to play a critical role for bridging between innate and adaptive immune system. CTCF is a DNA-binding protein composed of 11 zinc-finger domains. The CTCF mediates long-range DNA interaction that has impacted on three-dimensional chromatin structure. He described that CTCF regulated the homeostasis of epidermal DC Langerhams and bone marrow primitive hematopoietic stem cells. He also presented their results on the role of CTCF in shaping the 3D chromatin structure landscape to regulate gene expression programs in DCs.

The next APCC conference, **APCC8,** is scheduled to be held at Namik Kemal University in Turkey in 2023.

Summarized by Professor Nam-Soo Kim at Kangwon National University, Korea.
He was the chairman of APCC7 Organizing Committee. He is currently a KEEPER of the APCC.

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.

ANEUPLOIDY AND MOSAICISM IN HUMAN EMBRYOS

In a seminal paper, which appeared in Nature Medicine (2009), Joris Vermeesch and his colleagues demonstrated that chromosome instability is common in human cleavage-stage embryos. The authors of a paper that appeared in Genome Research (2020) have used a different approach on a sample of 74 human embryos and have reached the same conclusions: mosaic aneuploidies are widespread, with 80% embryos harboring at least one putative aneuploid cell. They were also able to infer that 55 (74%) embryos possessed mitotic aneuploidies and 23 (31%) embryos possessed meiotic aneuploidies.

HUMAN CHIMERAS

A recent review on *Natural Human Chimeras* (https://doi.org/10.1016/j.ejmg.2020.103971) puts a spotlight on a long known but rarely reported phenomenon. The term chimera, borrowed from Greek mythology, refers to individuals with two genetically distinct cell lines. The author, Madan, begins with high profile media reports of two women and a man whose parentage was disputed based on the 'irrefutable' DNA evidence. Further studies showed that DNA profiles from some tissues of the parents did match those of the children. All three turned out to be chimeras, each was made up of two zygotes of the same sex and each had a normal phenotype. What happens if the two fused zygotes are of different genetic sex? A review of 50 sex-discordant chimeras revealed that most were discovered because of abnormalities of sexual development. However, about a third were discovered by chance and had a normal male or a female phenotype, including fertility in some.

The author gives an overview, with illustrations, of the various ways in which chimeras can arise involving four or three gametes. Origins of individuals with gynogenetic or androgenetic cell lines and of those with diploid and triploid cells are also illustrated. Also, the various types of twins that are intermediate between the classical identical and non-identical are discussed.

Blood chimerism caused by transfusion across the fused placentas in dizygotic twins can last into adult life. Using examples from the long known twin-to-twin transfusion in cattle and marmoset monkeys, the author asks whether more than blood cells, for example germ cells, can be transferred in humans, as has been shown in marmosets.

Relatively few human chimeras have been reported in nearly 70 years since the first discovery. As most, including those in serological laboratories, have been discovered by chance, it has been suggested that chimeras may be more common than hither to thought. If that is so, the reliability of DNA tests comes into question with many medical and social implications. Further multidisciplinary research is required to address the many unanswered questions.

STRUCTURAL VARIATION: AN UPDATE

Since the first draft of the human genome published in 2001, there have been five new releases; the last being in 2013 (hg38). There is, however, still room for improvement and corrections. The main problems have been the variation that exists among different populations

and the difficulty in assembling complex duplicated regions. Recently, two main papers have addressed these issues. One is dedicated to the assembly of regions rich in segmental duplications (Nature Methods). The second is the largest study on the structural variations (deletions, duplications, insertions, and inversions) present in the different human populations (Cell), including those thought to be introgressed from the Neanderthals. It represents a more complete worldwide catalogue of structural variants present in human populations, obtained by studying nearly 1000 individuals from 54 diverse worldwide populations. The implications of these variants in gene expression and gene selection are also considered.
A nice comment/summary can be found in Trends in Genetics.

NEXT GENERATION MAPPING: KARYOTYPING REVIVAL WITH MOLECULAR BANDING

Since its first use in 1959 to unravel trisomy 21 (Down Syndrome), karyotyping has been the single pan genome method of analysis to decipher both numerical and structural abnormalities of the genome, balanced as well as unbalanced.
Its biggest drawback i.e. lack of sensitivity has been addressed by numerous improvements, almost one every 10 years: banding techniques in the 70s, high resolution banding technique in the 80s, FISH in the 90s, comparative genomic hybridization (CGH) in the beginning of the new century, and finally microarray analysis (either based on CGH or SNPs) in the 2010s. All of these improvements led to largely improved sensitivity, with increasing knowledge of cryptic rearrangements (nowadays called CNVs: Copy Number Variants). However, none of them could supersede the old Karyotype because of one or more of several limitations. It was either not a pan genome analysis method (FISH) or there was loss of paramount topographic information on the location of the abnormal segments (CGH or SNP arrays). Even the rise of various applications of massively parallel sequencing techniques, once a promise of an all-in-one tool for genetic analysis, was hardly convincing as a soon-to-become replacement for visual inspection of the chromosomes. This was because of the low sensitivity and specificity of current bio informatics tools for the detection of structural variants (SVs) and inherent limitation of short-read sequencing to overcome the preferential location of many breakpoints in difficult to sequence, repeat rich regions.
However, in keeping with the ‘once every decade’ path of innovation in cytogenetics, a new promising method has emerged; it could replace both Chromosome Microarray Analysis (CMA) and the karyotyping in patients referred for various reasons.
This technology, named Genome Optical Mapping (or NGM for Next Generation Mapping as opposed to NGS for Next Generation Sequencing), relies on the imaging of very long DNA molecules that have been labeled at specific sites after linearization in a microfluidic device. Without any sequencing (which may in some cases be an advantage by limiting the amount of information that one is not looking for), the labels on the DNA molecules are used as a molecular banding to reconstruct the genome by recognizing the identical patterns on the millions of molecules analyzed.
This paper which was recently published in BioRxiv https://www.biorxiv.org/content/10.1101/2020.07.15.205245v1.full addresses for the first time the performance of the Bionano’s optical mapping technology to decipher known chromosomal abnormalities previously identified with either karyotyping or CMA. Several teams were involved in this large study which shows a 100% concordance between standard clinical technologies and optical mapping. In some cases that were also analyzed with whole genome massively parallel sequencing technique, Bionano’s optical mapping could even find new, unforeseen

rearrangements or reveal very complex abnormalities that had been overlooked by CMA or NGS.
Further blind studies are required to test this approach against the current state-of-the-art techniques. However, it already holds the promise of becoming a first tier test for both Intellectual Deficiency / Developmental Disorders and reproductive disorders, although it may not be able to totally eclipse the old karyotype because of its inability to recognize breakpoints located in large heterochromatic regions (for example, whole-arm translocations and Robertsonian translocations).

NONCODING VARIANTS

When looking for the basis of a human disease, it is the exons that usually come under scrutiny. However, the ENCODE has revealed that a relatively large proportion of the noncoding human genome is functional. This review in Trends in Genetics deals with genetic diseases, some Mendelian, caused by noncoding variants.

NEANDERTHAL IN ICELANDERS

Kari Stefansson has published several human population studies on Icelanders. This time his paper in press in Nature examines the legacy of Neanderthals in phased genomes of 27,566 Icelanders. 14.4 million putative archaic chromosome fragments were identified. Of these, 84.5% are from Altai or Vindija Neanderthal, 3.3% are from Denisovan and 12.2 % are of unknown origin. The paper addresses the complex problem of introgression from archaic genomes, given that we have only three archaic individuals sequenced at high coverage, while the phenomena of introgression derive from a mixture of populations. These and related topics are well covered in this article.

LOSS-of-FUNCTION GENES

The 1000 genomes project has revealed 252 loss-of-function (LoF) genes occasionally found in homozygous state. Subsequent studies listed up to 2641 of these genes! These genes are apparently dispensable, very likely because of redundancy. This PNAS paper analyzes 190 of these genes with a relatively high frequency in at least one of the 5 studied populations. Many of these genes, quite expectedly, are olfactory receptor genes that may be undergoing pseudogenization. But, surprisingly, the paper notes that there may be positive selection for the loss of function of some genes and that this could be advantageous for human survival via an increased resistance to infectious diseases. Authors of a recent paper from a Chinese lab have shown that what nature does slowly can be done a lot more quickly in the laboratory by using gene editing technology. By knocking out both copies of two genes involved in viral infections, they have produced pigs that are now immune to some viral diseases.

Note: In mouse ‘knockout experiments’, the function of both copies of a gene is disrupted; study of the effect of the absence of the gene can give an insight into its function. In humans, function of genes can be similarly studied in individuals who are homozygous for a LoF mutation. Such mutations are more likely to be found in offspring of consanguineous marriages. Not surprisingly, a large number of the LoF genes listed by the study reported above, have been discovered in populations with a high rate of consanguinity. The authors of a Nature paper entitled “Human knockouts and phenotypic analysis in a cohort with a high rate of consanguinity”, have performed their study in Pakistan, where ~60% of the marriages are between first cousins.

FEMALE INFERTILITY - TWO NEW AUTOSOMAL GENES

Two papers in the July 2, 2020 issue of the American Journal of Human Genetics provide evidence for two novel, autosomal recessive causes for female infertility in humans. Both were identified in patients with normal menstrual cycles and multiple unsuccessful IVF attempts. Both papers originate from the same collective of fertility clinics and reproductive research institutes in Shanghai and other cities in China, and both demonstrate the power of Whole Exome Sequencing (WES) in the identification of genes underlying monogenic disorders of human reproduction.

First, Zheng et al. identified homozygous inactivating variants in the B cell translocation gene 4 (*BTG4*) in females from different consanguineous families. Oocytes from the affected females could be fertilized but failed to cleave. Studies of gene expression in the zygotes from these patients showed that hundreds of distinct maternal mRNA species failed to become degraded, thereby providing an explanation for the zygotic cleavage failure (ZCF) phenotype.

Second, Zhang et al. detected compound heterozygous and homozygous pathogenic missense variants in the Thyroid hormone receptor interactor 13 (*TRIP13*) gene in infertile female patients who had oocyte meiotic maturation arrest. In the mouse, TRIP13 serves to complete meiotic recombination by removing HORMAD2 from synapsed chromosome axes. In lymphoblastoid cell lines of the patients an abnormal accumulation of HORMAD2 was observed. Injection of wild type, but not of mutant *TRIP13* cRNA into HeLa cells could prevent HORMAD2 accumulation. Injection of wild type *TRIP13* cRNA into oocytes of one affected female resulted in completion of oocyte maturation as demonstrated by first polar body extrusion, successful fertilization and development up to the blastocyst stage. These observations may indicate novel therapeutic treatment options for oocyte maturation arrest.

BAR CODE... IN THE BRAIN

There are many situations in nature where an organism needs to produce a large genetic variability. In the unique case of the olfactory receptor (OR) genes, this has been achieved, during evolution, by multiplying the number of OR genes. There are, indeed, more than 800 OR loci, counting genes as well as pseudogenes.

Somatic recombination generates an infinite amount of diversity required for the production of antibodies.

Genetic diversity is also required for self/non-self-recognition. The Major Histocompatibility Complex (MHC) is composed of about 30 genes, but each locus is polymorphic, thus ensuring a very large variability. Plants have developed self-incompatibility to avoid self fertilization or inbreeding. This feature of plantas does not always suit Humans, who have have attenuated this incompatibility by domestication. This Youtube documentary on almond pollination in California will give you a better understanding of why this is so.

Nature (not the journal) reaches the same goal in many different ways. Neurons have to connect to other neurons, not to themselves. The alternative splicing of the many exons of the *DSCAM* gene in *Drosophila* neurons potentially generates more than 38,000 *DSCAM* isoforms. This molecular diversity could contribute to the specificity of neuronal connectivity and thus self-connection avoidance (see Nature).

A paper which appeared in Nature (August 27) reports that the 118 distinct classes of *Caenorhabditis elegans* neurons are specified by unique combinatorial expression of the102 homeobox genes. In other words: one can precisely define the class of each neuron by deciphering the bar code expression of the homeobox genes.

Variability in a broad biological context is inherent to the complex life on Earth. Primordial multicellular organisms had the potential of transmitting tumors to almost identical individuals in a population. Genetic diversity, to ensure non-self-recognition and rejection, was

needed. Sexual reproduction, which enhances variability among individuals, was a big step towards accomplishing this task. Indeed, since its appearance ~1.2 billion years ago, sexual reproduction has rapidly and almost completely spread among multicellular organisms. The best indirect proof of this hypothesis is represented by the marsupial Tasmanian devil. This species shows a paucity of MHC diversity due to the high rate of inbreeding. As a consequence, the non-self-rejection is very poor. Indeed, two papers reported transmission through bites of a mouth cancer among these marsupials (PNAS, PNAS).

THE X-CHROMOSOME TELOMERE-TO-TELOMERE, NO GAPS

The initial human genome reference map was full of gaps, that is, "holes" in the sequence that were impossible to clone in bacteria. In subsequent years most, but not all, gaps were filled (see hg38); the centromeres remained as gaps. Centromeres are full of repetitive sequences and are highly polymorphic in the population. Then new technologies that were able to yield ultra-long (greater than 100 kb) reads became available. The fully haploid cell line CHM13hTERT also proved to be very useful in patching the gaps. This Nature paper reports the first human chromosome, the X chromosome, the sequence of which extends from pter to qter with no gaps.

NATURAL SELECTION VS. NEUTRAL EVOLUTION

What is the contribution of each of these two processes (natural selection and neutral evolution) to the evolution of genomes?

In 1983, in a book called The Neutral Theory of Molecular Evolution, M. Kimura summarized his theory that was first published in Nature in 1968. He claims that "*the overwhelming majority of evolutionary changes at the molecular level are not caused by selection acting on advantageous mutants, but by random fixation of selectively neutral or very nearly neutral mutants...*".

As far as natural selection is concerned, there are several examples of strong selection which very quickly spreads the mutation in the population. The lactase persistence in some population is the most striking example (see Schlebusch et al., 2012). In many situations, however, it is not easy to determine if a DNA trait was under selective pressure or originated from neutral evolution.

With respect to this debate, Hunter B. Fraser (Stanford) has just published a paper in PNAS. He says "*I introduce an approach to detecting selection* (on quantitative traits) *that makes minimal assumptions and only requires phenotypic data from ~10 individuals*"; And concludes: "*In sum, this test is applicable to phenotypic data from almost any genetic cross, allowing selection to be detected more easily and powerfully than previously possible.*".

Moreover, with regard to the above mentioned question, his work reaches an important conclusion: "*Applied to empirical data, the test reveals widespread selection in both domesticated and wild species*".

SPERM GENOMES

The first human aneuploidy, the trisomy of chromosome 21 was described by Lejuene in 1959. Subsequent research showed that about 50% of spontaneous abortions have a chromosome anomaly. Most of these were trisomies; monosomies, theoretically expected to equal trisomies, were absent (with the exception of a special chromosome, the X). The obvious hypothesis was that monosomies were incompatible with normal development of the early embryo. Researchers were eager to investigate the chromosome constitution of embryos at very early stages. The group of J.Vermeesch has successfully analyzed human cleavage-stage embryos, disclosing their high

chromosome instability. Aneuploidy in sperm cells has been studied in the past using different approaches (sperm–hamster oocyte fusion system; FISH).
Since then, technology has made it possible to sequence the genome of single cells. Using an ingenious procedure Bell et al. (Nature), have fully sequenced 31,128 human sperm and their 20 donors. They found 787 whole-chromosome aneuploidies and 133 chromosome arm-scale gains and losses (2.5% and 0.4% of cells, respectively). They also obtained interesting data on correlation between crossovers and non-disjunction.
In evaluating these results in the wider context of human reproduction, one has to remember that female meiosis, where the rate of aneuploidy increases with maternal age, is more prone to non-disjunction. As mentioned above, chromosome gain and loss frequently occur also after fertilization, in cleavage-stage embryos

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FISH and CHIPS
Oops! FISH and CRISPR

The sequence specificity of the CRISPR-Cas9 technology has inspired its exploitation to reveal specific sequences by coupling the CRISPR machinery with a fluorochrome. Several papers have been published on this topic since 2013. The technology has proved to be problematic, but the continued improvements in the system were promising. The paper by Chaudhary et al. (2020) focuses mainly on suppression of the background fluorescence and claims that the technique can be used with a conventional fluorescence microscope (the one familiar to cytogeneticists).
The procedure still faces some limitations: the requirement for PAM sequences and the need for a minimum number of repeats. This means that unique sequences are not in its range for now. On the other hand near-PAMless engineered CRISPR-Cas9 variants, for instance, have already been reported last April in Science. The technology, anyway, deserves attention because of its great potential advantages: it requires neither DNA denaturation, nor a hybridization (usually long) step, nor a large DNA clone to be used as a probe.

ONLINE KARYOTYPING

Online karyotyping for beginners is just one mouse click away:
https://ilias.hhu.de/ilias/goto.php?target=cat_836019&client_id=UniRZ

Harald Rieder (and his colleagues) at the Institute of Human Genetics of the Heinrich-Heine-University in Dusseldorf Germany, have developed a site for an online course on karyotyping. A set of normal G- and R-banded male karyotypes is provided to get familiar with normal chromosome morphology. It also includes chromosome sets from cells with a numerical or a structural aberration. Students can organize the chromosomes into a karyogram online and check whether their classification is correct by using a template with the final complete or partial karyogram. A karyotype according to the International System for Cytogenetic Nomenclature could also be provided as the program is developed further.

TO WHAT EXTENT DOES GENE DOSAGE MATTER?

Several technologies allow the detection of copy number variations (CNVs) such as gene deletions and duplications using well-known procedures in Cytogenomics laboratories. These anomalies cause dosage alterations of the genes annotated to the affected region. In the case of deletions, hemizygosity can lead to a wide range of effects, from abnormal phenotypes (haploinsufficiency) in the worst scenario to the absence of any phenotypic effects in the best. Besides differences in penetrance, time in life when effects appear is also highly variable, from congenital defects to adult-onset diseases.

In this paper from Trends in Genetics, the author has performed an outstanding review of the phenotypic effects of 238 non-recurrent gene deletions affecting chromosome 18. As expected, the most frequent result is the lack of clinical effects suggesting that dosage insensitivity and dosage compensation are the most frequent situations. Nevertheless, hemizygosity also produces a wide spectrum of effects including highly penetrant phenotypes (19 genes), low penetrance phenotypes (9 genes), conditional effects (49 genes) and risk factors for polygenic disorders (15 genes). All these data are summarized in Table 1 and could be useful for accurate and predictive clinical management of the affected patients.
The author also provides an interesting discussion about the molecular mechanisms behind haploinsufficiency. Factors based on the presence of conserved gene sequences, the alteration of topologically associated domains (TADs), the presence of homologous genes, and the functionality of dosage sensitive genes are discussed. Finally, the paper also addresses the association between variable penetrance, gene dose compensation and epigenetic modifications.

COVID-19 AND... NEANDERTHALS

One haplotype of the locus mapping at chromosome 3: (chr3:45,859,651-45,909,024 hg19) represents a genetic risk factor for COVID-19 infection. Zeberg and Pääbo, in their paper in Nature, pinpoint that this variant haplotype is one of the many genomic segments introgressed into Eurasians from Neanderthals. Indeed, the variant is absent in Africa. The frequency in Eurasia varies substantially, according to a supposed positive or negative selection. A positive selection occurred, very likely, in Bangladesh (protection from other infections?), where it is present, at least in heterozygosity, in about 63% of the population. The impact of the variant is better appreciated in UK where, in similar environmental conditions, individuals of Bangladeshi origin have about two times higher risk to die from COVID-19 than the general population.

REPEAT EXPANSIONS - FRAGILE SITES - AND AUTISM SPECTRUM DISORDER (ASD)

Repeat expansions have been associated with specific diseases, the Fragile X syndrome being the prototypical example. Many of them are associated with fragile sites. The variability of these loci is difficult to detect using short read genome sequencing. Trost et al. (Nature 2020), exploiting newly developed detection methods, have examined 17,231 individuals (1,558 affected) from ASD families. 2,500 control individuals were also sequenced. The study identified 31,793 repeat domains. Many of these correlate to known fragile sites and 2,588 of them lie in gene domains. The authors found a significantly higher prevalence of rare repeat expansions (23.3%) in children with ASD compared to the control population (20.7%). These rare tandem repeat expansions represent a collective risk of ASD of 2.6%.
Unfortunately, technical limitations still persist, the detection threshold being 150 bp.

GENETICS AND ODOR PERCEPTION

A large battery of Olfactory Receptor (OR) genes allows us to distinguish many odors (and tastes). The OR family is the biggest gene family in mammals. Counting genes as well as pseudogenes, there are, indeed, more than 800 OR loci in man and more than 4000 in the elephant (Genome Research). Many of them, however, are non-functional. In man, more than half of them are non-functional pseudogenes. However, OR population genetics has shown that two thirds of human OR loci segregate between an intact and inactivated alleles (BMC Genomics), indicating that humans have a highly personalized repertoire of ORs. A recent

paper in Current Biology, by the Stefansson's group, adds a further inter-individual diversification. The authors analyzed 9,122 Icelanders and replicated their results in a separate sample of 2,204 individuals. They found that the difference between two alleles is not always as simple as active or inactive. The authors identified variants of three genes associated with odors of licorice, cinnamon and fish, where the same odor is differently perceived and named. Thanks to the *TAAR5* gene, many of us perceive the odor of rotten fish as disgusting. Individuals carrying a variant of this gene do not perceive it at all or positively describe it as potatoes, caramel or rose.

GENES AND BEHAVIOR: OVERNIGHT SOCIAL ISOLATION AFFECTS GENE EXPRESSION IN THE SONGBIRD'S FOREBRAIN

In the September 22 issue of the PNAS (USA), Julia George and co-workers from London, St. Andrews (UK), Urbana (Illinois, USA) and Seewiesen (Germany) show that social isolation of zebra finches leads to rapid alterations of gene expression in the auditory forebrain.

Similar to humans, songbirds also communicate using learned vocalizations. The *FOXP2* gene was the first gene in humans shown to be essential for speech and language development. Also in birds this gene has a function in the learning of vocal communication, and the highly social zebra finch (*Taeniopygia guttata*), which had its genome sequenced in 2010, has emerged as a model organism to study the neurobiology of vocal learning, including its genetic basis (see Cold Spring Harbor Protocols). Some 55 genes, including *FOXP2*, have similar patterns of gene expression during vocal learning in humans and in zebra finches.

Using high-throughput RNA sequencing techniques, genome-wide methylation assays by bisulfite sequencing and in situ hybridization, one can study how the exposure to auditory signals influences gene expression in the bird's forebrain. George et al. applied these methods to show that genes annotated for axonal guidance and neutrophin pathways are repressed when the bird is isolated overnight in a sound attenuating chamber, and that this is accompanied by changes in DNA methylation.

Previous studies have shown that prolonged isolation has negative effects on brain and behavior in social organisms such as humans and birds. Now, George et al provide a dramatic example of environmental effects on brain gene expression by showing that overnight isolation is sufficient to trigger epigenetic changes affecting gene activity in a higher integrative center of the songbird's brain.

EXOME SEQUENCING FOR NEWBORN SCREENING

In a recent paper in the AJHG, Roman and her coworkers blindly sequenced the exome 106 children: 17 with inborn errors of metabolism, 28 with hearing loss, and 61 healthy newborns, to simulate a newborn screening. The study detected a medically actionable variant in 4 of 106 newborns that would have been missed by the standard newborn screening procedure. They also found that there were on average 1.8 reportable carrier variants per child. These findings per se are not novel, but the comparison to the standard newborn screening is new. The paper provides us with a glimpse of what we can expect if exome sequencing were to be used for newborn screening programs as a diagnostic tool. The authors stress the importance of finding a balance between increasing sensitivity, to maximize case finding, versus establishing stringent thresholds to reduce false positives.

CHROMOSOME INSTABILITY AND TUMOR EVOLUTION

In 2011 "Omics" of single cells made it possible to analyze clonal evolution in cancer using methods typically used in population genetics

(Nature). On March 29, 2013 Science dedicated a special section to this topic, titled "The downside of diversity" in which Charles Swanton (Crick Institute, London) was central stage. In the 5 November 2020 issue of Nature, Charles Swanton and his group have published a paper analyzing the diversity in depth, chromosomal diversity in particular, as a driving force of tumor evolution.

ANEUPLOIDY HELPS CANCER. HOW?

Cancer is an uncontrolled cell proliferation. Aneuploid cells are slow dividing, yet aneuploidy is a hall-mark of cancer, especially after chemotherapy. How can that be? J. M. Reploglea et al. (PNAS) have found experimental proof of an explanation which is a simple and intuitive. Chemotherapy drugs act on proliferating cells; aneuploid cells lag behind in G1 and remain safe. The slow proliferation rate and the great variability of aneuploid cells ensure high drug resistance.

RESTOCKING, HETEROZYGOSITY, AND THE "PURE RACE"

The cover of the Science issue of 27 November is dedicated to the desert tortoise with a headline 'Variation Matters'. An article by Scott et al. deals with restocking of Mojave Desert tortoises (*Gopherus agassizii*s). The authors analyzed the variables of the 166 individuals used in restocking, in search of the best predictor of success of adaptation to new environments. The study suggests a relatively simple indicator of the likelihood of survival of the translocated individuals : heterozygosity. Heterozygosity of the individual obviously reflects the variation in the population.
The myth of "pure race" is, from a genetic point of view, a stupidity.

FAMILY RELATIONSHIPS AND SOCIAL ORGANIZATION IN BELL BEAKER CULTURE COMMUNITIES

In a remarkable cross-disciplinary study of burial sites at two Copper Age Bell Beaker culture settlements in South Germany, Sjögren et al. (PLoS ONE, November 16, 2020) provide a detailed reconstruction of kinship patterns and social organization.

The study involved archeologists, historians, anthropologists and molecular biologists and was coordinated by Kristian Kristiansen (Gothenburg, Sweden) and Volker Heyd (Helsinki, Finland). The work is based on two burial sites, in Irlbach and Alburg, close to the Danube River and 17 kilometers apart. The sites are typical for the Bell Beaker culture, which was predominant almost all over Europe during 2750-2000 BC.

Anthropometric analysis of the skeletons enabled the determination of sex and age at death. Strontium and oxygen isotope ratios in tooth enamel were determined to find out if individuals grew up at similar or different geographical locations. This, combined with the sequence analysis of DNA fragments from bones provided evidence for a patrilocal society in both communities. Whereas males shared a single Y-haplotype there was a much higher maternal genetic diversity, with 23 mitochondrial haplotypes in 34 individuals. The reconstructed pedigrees were consistent with monogamy combined with exogamy, as six of eight non-locals were women.

Thus, successive generations of males remained in these communities and women came from elsewhere. In this way property such as farming grounds could be conserved and inbreeding was prevented. The single Y-haplotype, with a Steppe-pastoralist origin from Eurasia, is still the dominant type in central and western Europe today.

NEW INSIGHTS INTO THE XY BODY FUNCTIONS

A key stage in meiosis is the synapsis of homologous chromosomes followed by exchange of genetic material to generate cross-overs. In mammals, when chromosomes fail to synapse, the unsynapsed segments are trans-criptionally inactivated by a process directed by the DNA Damage Response pathway (DDR). In heteromorphic sex chromosome systems, this process leads to the formation of a riveting nuclear structure, the XY body, which is the cytological manifestation of the meiotic sex chromosomes inactivation (MSCI).

A recent paper in Current Biology delves into the molecular events leading to the formation of this structure and its functionality. Using a defective MSCI mouse model, the authors demonstrate that, besides the well-known ATR induced H2AX phosphorylation at Ser139, phosphorylation at Tyr142 is also required for the initiation of meiotic sex chromosome inactivation and the formation of the XY body. More intriguingly, the observation of persistent DDR foci on autosome axes from defective MSCI mice has led the authors to propose that in the early pachytene stage, XY chromatin sequesters the DDR signaling from the autosomes to the sex chromosomes, a process essential to the progression of germ cells through meiotic prophase I.

As Mary Ann Handel states in her comment about this article "… the authors propose a novel role for MSCI, positing that by attracting and sequestering DDR proteins, it serves a checkpoint or licensing function. … The idea that MSCI and XY body formation together form an essential pacing mechanism for progress through meiosis is novel and exciting, and the finding that the XY body specifically sequesters proteins builds on merging views of the physical nature of heterochromatin". Without a doubt, Namekawa's article opens new and exciting scenarios for future research in this field.

NEVER ENDING HUMAN GENOME PROJECT

Lee et al. (BMC Biology) have used a new software (InserTag) to analyze the sequence data obtained from the 2535 individuals of the 1000 Genome Project in search of missing sequences (>50bp) in the human reference assembly (hg19). They found 1696 non-reference insertion variants, re-classified as (i) retention of ancestral sequences* or (ii) novel sequence insertions, based on the ancestral state. Individuals had, on average, 0.92-Mbp sequences missing from the reference genome. 92% of the variants were common (> 5%), and more than half were major alleles (!).

* "retention of ancestral sequences" means that they are present in non-human hominids (chimpanzee, gorilla, orangutan).

ALTERNATIVE TELOMERES

Different organisms use different solutions for the same problem. DNA replication implies that the ends of the chromosomes shorten at each cell replication. The vast majority of eukaryotic organisms use a repeated TTAGGG (or variant) sequence at the end of the chromosome to solve the problem. A portion of these sequences are truncated at each cycle*, but the proximal genes are protected. Diptera (*Drosophila*, for instance) use long terminal repeats (LTR) as an alternative solution for the telomere shortening problem.

A paper in BMC Biology reports that the tapeworm (*Hymenolepis microstoma*) uses centromeric sequences as a protecting cap at the end of the chromosomes.

* The protection is not indefinite because telomerase, which regenerates the length of the telomeres in embryonic and germ cells, is not expressed in many somatic cells (cell senescence).

RECOMBINATION FAILURE IN HUMAN OOCYTES

Meiotic errors are a hallmark of human gametogenesis, leading to an extraordinary high level of aneuploidies in conceptuses as compared to most other species. Specific maternal-age associated increase in aneuploidy rate is the only well described risk factor which has justified numerous studies to understand the molecular basis for this phenomenon. A second pathway to aneuploidy was revealed by previous studies on recombination during meiosis showing a link between altered crossing over and non-disjunction of homologues: almost 50% of trisomy 21 children had no crossing over between the two chromosomes 21 that failed to disjoin during meiosis.

In a paper which appeared in Am J Hum Genet, the team of Terry Hassold and Patricia Hunt addresses the question of how important is the absence of exchange between homologues in human meiosis. For the first time, they were able to analyze a large population of female meiotic cells, 7396 oocytes from 160 fetal ovaries, and showed a very high level of "exchangeless" chromosomes, up to 7%-10% of all analyzed cells. In line with previous observations on cell division check points in female versus male meiosis, the authors observed a ten-fold increase in the incidence of exchangeless chromosomes in oocytes versus spermatocytes.

As expected, their results show that most events of absence of crossing over are concentrated on small G group chromosomes. However, in contrast to previous studies, the authors did not observe a direct correlation between genome-wide size of the synaptonemal complex and the risk of exchangeless chromosomes, leading to the conclusion that the size of the synaptonemal complex may not be the main determinant for absence of crossing over. Rather, the large inter-individual variation in the frequency of exchangeless chromosome seems to be associated with overall level of meiotic recombination.

Exploring the reasons for inter-individual variation in recombination rate, the authors did observe a surprising correlation between maternal age and the number of recombination foci in the oocytes of the female fetuses; they suggest the existence of a grandmaternal effect on recombination where the age of a pregnant woman affects the recombination profile of her daughter's oocytes.

See also: https://www.ashg.org/publications-news/ashg-news/inside-ajhg-with-terry-hassold/

THE EFFECTS OF COMMON STRUCTURAL VARIANTS ON 3D CHROMATIN STRUCTURE

The 3D chromatin structure consists of chromatin loops and Topologically Associated Domains (TADs), which control physical interactions between genes and regulatory sequences. The cohesin protein complex and the CCCTC-binding factor CTCF localize to the boundaries of TADs. Structural variations affecting such boundaries disrupt gene-enhancer contacts leading to ectopic interactions between promoters and non-coding DNA and have been shown to cause skeletal abnormalities such as polysyndactyly, and craniofacial abnormalities. By Chromatin Conformation Capture (Hi-C) sequencing of 19 lymphoblastoid cell lines from the 1000 genomes project Shanta and colleagues (2020) studied the effects of common structural variations on 3D chromatin structure. They found distinct signatures depending upon the molecular type of the structural variation. Deletions, which remove TAD boundaries and cause TAD fusion would be rare since they may be under negative selection in the general population. Large inversions (e.g. at 8p23.1) have effects on chromatin interactions that span the inversion breakpoints, and are able to affect 3D structures within a 2 Mb distance.

HOW TO SELECT A SHORTLIST OF GENETIC MARKERS FOR MALE INFERTILITY?

In reproductive genetics, the application of high throughput techniques such as Whole Exome Sequencing has extended the number of pathogenic gene variants associated with male infertility, opening the debate about the utility of the clinical implementation of diagnostic gene panels. But, which genes should we include in these panels to obtain a high diagnostic yield and high specificity and sensitivity?
So far to date, the most common strategy used for gene selection has been literature search in the MEDLINE-Pubmed database. Nevertheless, one of the limitations of such a strategy is the difficulty in classifying and in selecting the genetic variants according to their degree of association with male infertility, that is, the difficulty of ordering them from the most to the least predictive.

This article published in *Cytogenomics and Genome Research* introduces an interesting alternative. Based on regression model analysis, the authors have developed a ranking of genes according to their potential as fertility biomarkers. The analysis takes as a starting point genes associated with male infertility in knockout mice (n=251) and genes selected from studies in infertile men (n=52). Both lists are further analyzed considering sequence gene conservation, testis transcription level, and their connectivity in a protein-protein interaction network. Using this strategy, the authors identify 9 genes with the highest predictive values suggesting their potential as candidates for becoming a part of a gene panel. Besides, additional genes with high values are also suggested, compiling a final list of 22 markers. Interestingly, the predictive value of the selected genes was validated using quantitative proteomics in spermatozoa from fertile and infertile patients, and differential expression was observed in 23% of the genes reinforcing their potential as infertility markers.

Although additional work should be done to validate the list provided in this article, some characteristics of this selection method (use of public database information, quantitative interpretation of the results, and the possibility to add new predictive variables) make it an interesting and novel approach.

PRESERVING CYTOGENOMIC COMPETENCE OF DIAGNOSTIC GENOME LABORATORIES IN THE AGE OF WHOLE GENOME SEQUENCING

The classical approach to suspected genetic disorders is karyotyping, which is limited in its level of resolution by the number of chromosome bands detected. Next generation sequencing, in its ultimate form of whole genome sequencing (WGS), which represents the patient's genome as a string of nucleotides, allows to overcome this resolution problem. Yet, eukaryotic genomes are not organized as a single string of nucleotides, but in chromosomes. Untoward changes in their structure account for a considerable part of the pathology encountered in clinical genetic laboratories. Precisely what structural or numerical change(s) have taken place determines the risk for clinical phenotypes, their prognosis and their recurrence risk. In their recent analysis Hochstenbach, Liehr and Hastings (Eur J Hum Genet) point to this inherent weakness of WGS and to the loss of awareness of this problem among clinical laboratory geneticists (CLGs) as became evident during evaluation of clinical genetic laboratories by External Quality Assessment schemes (EQAs). While interpretation of WGS data becomes increasingly automated, thus reducing the need for human intervention, analysis of structural and numerical genome changes continues to require the “human eye”. The demand for cytogenomic competence will only rise in the near future, and thefore needs to be preserved during the education of CLGs.

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E.C.A. News

- Due to the Corona Pandemic, the Board intends to hold the 13th European Cytogenomics Conference on-line this year, starting on the traditional date, Saturday 3 July 2021.
- As required by the E.C.A. statutes, a physical General Assembly with Board elections is planned in the second half of 2021.
- The Nîmes Course will be organized on-line 1-13 March 2021; the Goldrain Course is at present scheduled on-site for 28 August to 5 September 2021.

E.C.A. Fellowships

- The **E.C.A.** offers two **Fellowships** for the following course:

  Goldrain Course in Clinical Cytogenetics
  to be held in Goldrain Castle (South Tyrol, Italy) 28 August – 5 September 2021.

- The fellowships **include the course fees and the accommodation** during the lectures in Goldrain but **do not include travel expenses**.
- Applications with CV, list of publications and a letter of support should be addressed to the course organizer. The Educational Advisory Council of the E.C.A. will select the candidates.

E.C.A. PERMANENT WORKING GROUPS (PWG)

PWG: CLINICAL AND MOLECULAR APPROACHES TO CYTOGENETIC SYNDROMES.

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PWG: MARKER CHROMOSOMES.

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Please remember that the sSMC homepage can now be reached at
http://cs-tl.de/DB/CA/sSMC/0-Start.html
and UPD/ heteromorphisms and M-FISH pages at
http://cs-tl.de/ and http://cs-tl.de/DB.html

The PWG had a meeting at the 2019 European Cytogenomic Conference in Salzburg. Two of the presentation there have been published in the meantime:
Slimani W, Jelloul A, Al-Rikabi A, Sallem A, Hasni Y, Chachia S, Ernez A, Chaieb A, Bibi M, Liehr T, Saad A, Mougou-Zerelli S. Small supernumerary marker chromosomes (sSMC) and male infertility: characterization of five new cases, review of the literature, and perspectives. J Assist Reprod Genet. 2020 Jul;37(7):1729-1736. doi: 10.1007/s10815-020-01811-9. Epub 2020 May 12. PMID: 32399795; PMCID: PMC7376793.

Barišić A, Finderle A, Petrović O, Vraneković J.
Bifid cardiac apex in Pallister-Killian syndrome: case report. medicina fluminensis 2020;56(2):189-192.

PWG: CYTOGENETIC TOXICOLOGY AND MUTAGENESIS.

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Writing this brief report has given us the opportunity as Coordinators to reconnect with members of this permanent working group after the long 'Covid19' hiatus. I am pleased to report that everybody is well and making progress in spite of the professional challenges and setbacks brought about by the pandemic. Disappointingly, many of the collaborations and networking propositions that had been explored during the last satellite symposium at ECA Conference in Salzburg, had to be shelved given the extraordinary circumstances. However, we are hoping in better times. Meanwhile, it was reassuring to find out that individual research endeavours within the group have persisted and work on chromosomal instability that was presented as preliminary at the satellite meeting in 2019 has been successfully taken forward. The excellent work presented by Isadora May Vaz, an early researcher from the Pontifícia Universidade Católica do Paraná in Curitiba in Brazil, on 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, has been recently submitted for publication. Radhia M'kacher (Cell Environment DNA damage R&D, Paris) and Eric Jeandidier (Groupe Hospitalier de la Région de Mulhouse et Sud-Alsace) have published their

beautiful work on the clinical utility of centromere and telomere staining followed by M-FISH to detect chromosomal instability for clinical purposes: https://www.fertstert.org/article/S0015-0282(20)30629-4/ fulltext; https://www.mdpi.com/2073-4425/11/5/475. Ivan Iourov (Mental Health Research Centre, Moscow, Russia), who had presented on somatic chromosomal mosaicism and instability in neuro-developmental diseases, has since published numerous articles that further ground the pioneering work on this extremely interesting topic carried out by the late Yuri Yurov and his team:
https://doi.org/10.3390/genes10050379;
https://doi.org/10.3389/fgene.2019.00892;
https://doi.org/10.1186/s13039-019-0467-8;
https://doi.org/10.1186/s13039-020-00488-0;
https://doi.org/10.3390/ijms21218328.
As I said, we hope in better times and very much look forward to our next meeting. Meanwhile best wishes for a healthy and serene 2021 to you all.

PWG: ANIMAL, PLANT, AND COMPARATIVE CYTOGENOMICS.

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PWG: CYTOGENETICS OF HAEMATOLOGICAL MALIGNANCIES.

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PWG: CANCER CYTOGENETICS, SOLID TUMOR STUDIES.

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PWG: QUALITY ISSUES AND TRAINING IN CYTOGENETICS.

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PWG: CYTOGENOMICS.

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Permanent Working Group on Prenatal Diagnosis, Salzburg, 2019

Being an area that is already so old (first karyotype obtained from chorionic villi in 1980s), chorionic villi diagnosis continues and even grows for those who work in prenatal cytogenetics, raising several problems both in the technical realization and in the methodology to be applied.

We decided to make an interactive section so that those who work in this area can express themselves. The questions that were presented were based on a previous survey that we made to five Portuguese cytogenetics laboratories (one in Vila Real, three in Porto and one in Coimbra) and to five European laboratories (Germany, France, Italy, Spain and England).
We present here the results of this interactive survey, after a short reminder of embryologic origin of the analysed cells, of the cytogenetic methods and of the European recommendations.

Embryology

Placenta derives from the very first cell differentiation during embryonic development, giving rise to the trophoblast at day 5. After contact with uterine mucosa, trophoblast cells further differentiate into Cytotrophoblast and Syncytiotrophoblast.
Cytotrophoblast cells replicate heavily and give rise to daughter cytotrophoblastic cells and to cells that fuse together to create and grow the syncytiotrophoblast. Proliferation of cytotrophoblast leads to the apparition of peripheral projections called primary villi, which are later invaded by mesenchymal cells derived from extra embryonic mesenchyme (secondary villi). These fibroblasts constitute the chore of the villi which are called tertiary villi when blood vessels penetrate the mesenchymal axis. This axis is surrounded by rapidly dividing cytotrophoblast cells and a thin layer of syncytiotrophoblast (non dividing) cells.
Mesenchymal cells of the villi axis derive from the endoblast, the second embryonic layer to differentiate from the inner cell mass. Hence, from an embryologic perspective, the extra-embryonic mesenchymal cells are closer to the embryo proper than are the Cytotrophoblastic cells which differentiate from the Trophoblast.

Cytogenetics

Chorionic Villi Sampling (CVS) is usually performed after 10 weeks of pregnancy.
Two cytogenetic preparations can be done on a chorionic villi sample to obtain a karyotype:

- Short-term incubation (direct preparation)
- Long-term culture

Short-term preparation uses an overnight incubation of the villi in culture media and takes advantage of the high cell division rate of cytotrophoblast to obtain metaphase spreads the following day. Two main disadvantages of the method are the lower quality of chromosome preparations (low resolution) and the possibility that a chromosome aberration detected in the Cytotrophoblast is not present in the fetus (placental mosaicism). The main advantages are the fast result and the absence of maternal contamination (maternal cells, if present, will not give metaphase spreads after one night incubation because of the low cell division rate).
Long-term preparation is used to culture the fibroblasts of the mesenchymal core of the villi, which derive from the differentiation of the foetal endoblast. The advantage is a better quality of chromosomes (same quality as obtained with amniotic fluid) and a better proxy to foetal karyotype. However, turnaround time to get a result is longer (usually 14 days) and maternal contamination is an issue if decidua have not been carefully removed.

ECA recommendations (2012)

The reference technique is long-term culture which minimizes the risk of misinterpretation of fetal karyotype. Direct preparation should never be used alone but always associated to a long-term culture as a confirmation. In cases where the sample size in inadequate for both short-term and long-term

preparation, the long-term culture should prevail, and the use of FISH or QF-PCR considered to detect the main aneuploidies.

Combination of both results (short-term and long-term preparations) helps in distinguishing between various types of confined placental mosaicism and foeto placental discrepancies.

Type I: only cytotrophoblast (short-term preparation) is abnormal

Type II: only mesenchymal fibroblasts are abnormal (long-term culture)

Type III: both lineages are abnormal.

Interactive survey during the Prenatal Permanent Working Group session

Several questions were asked and answered interactively by the audience during the session, covering three main areas:

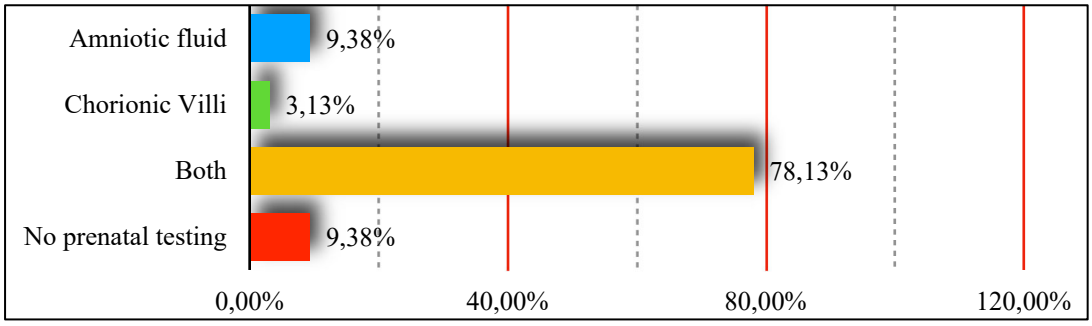
A) Prenatal testing organisation: what kind of prenatal sampling is routinely used, which are the main reason for referral, what is the usual amount of villi obtained

B) Cytogenetic workflow for chorionic villi samples: which method (direct preparation and/or long-term culture) is used, how many cells are analysed, which molecular methods are used to complement karyotyping of chorionic villi

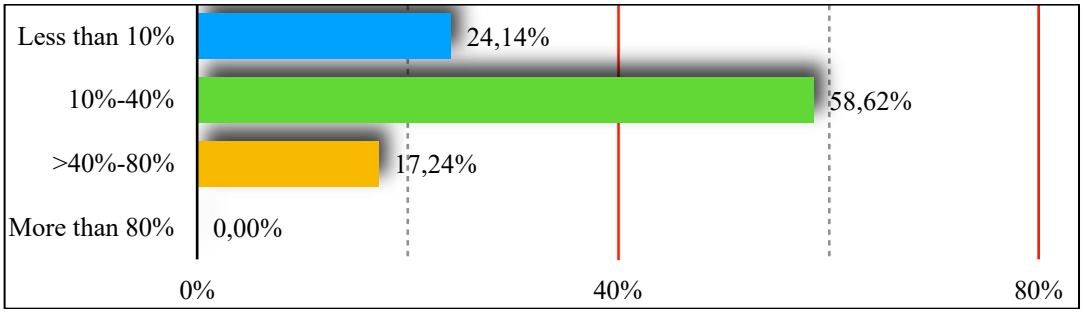
C) Guidelines

A) Prenatal testing organization

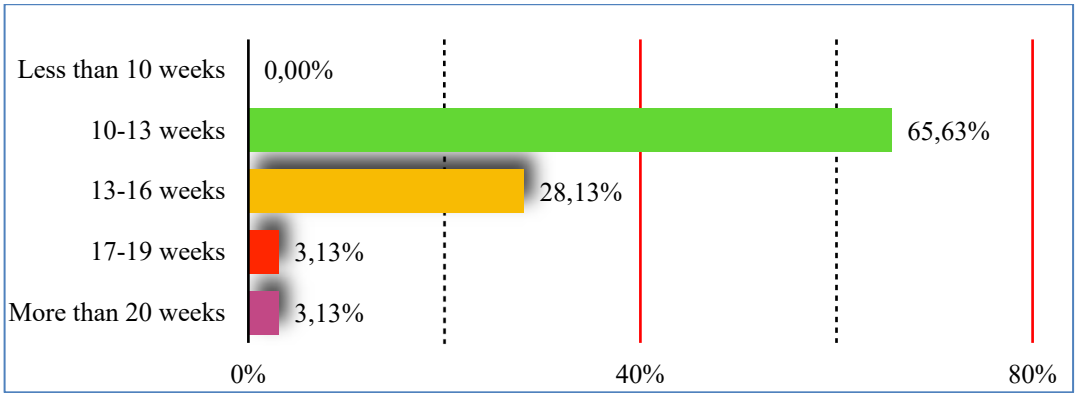
1. What is/are the sample(s) for prenatal testing that your laboratory receives?



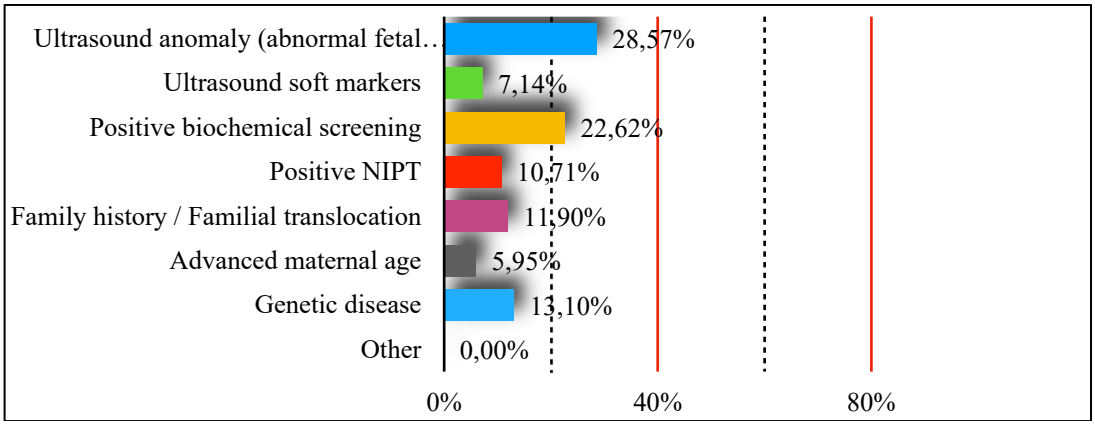
2. What is the incidence of chorionic villi samples in your laboratory



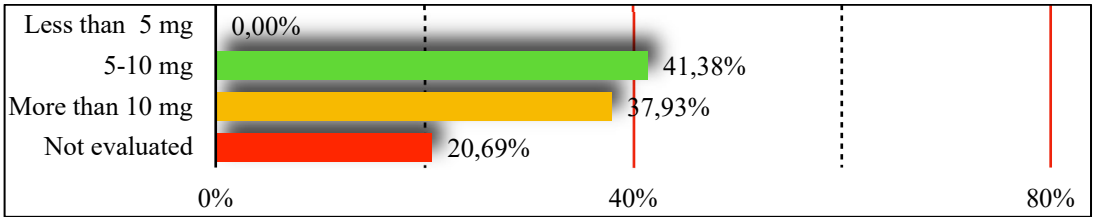
3. Regarding the weeks of gestation, the majority of the chorionic villi samples are collected when?



4. According to your experience, what are the 3 principal reasons for referral using chorionic villi?

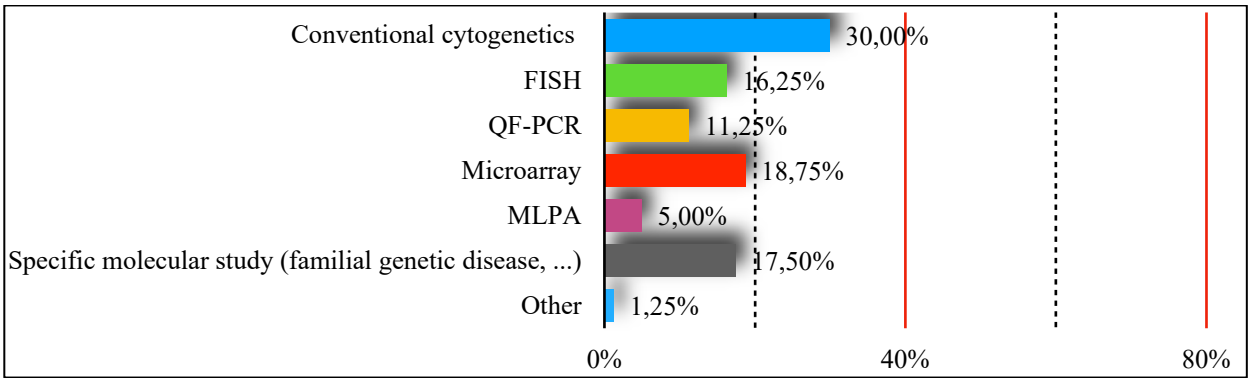


5. What is the most common amount of chorionic villi sample (mg) that you receive

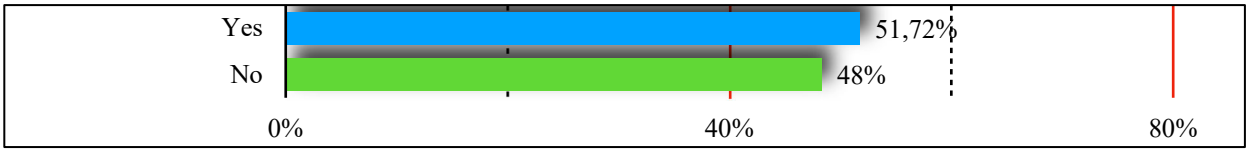


B) Cytogenetic workflow for chorionic villi analysis

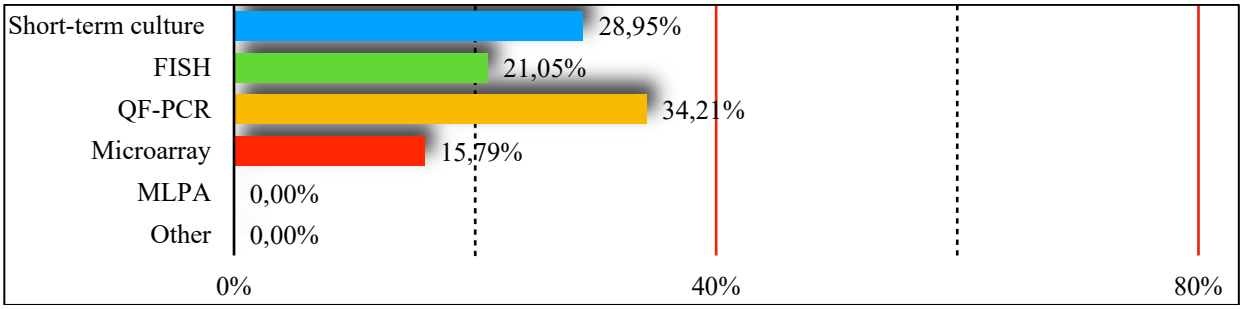
6. Per routine, which approach do you perform on chorionic villi samples? (you can select more than1 option)



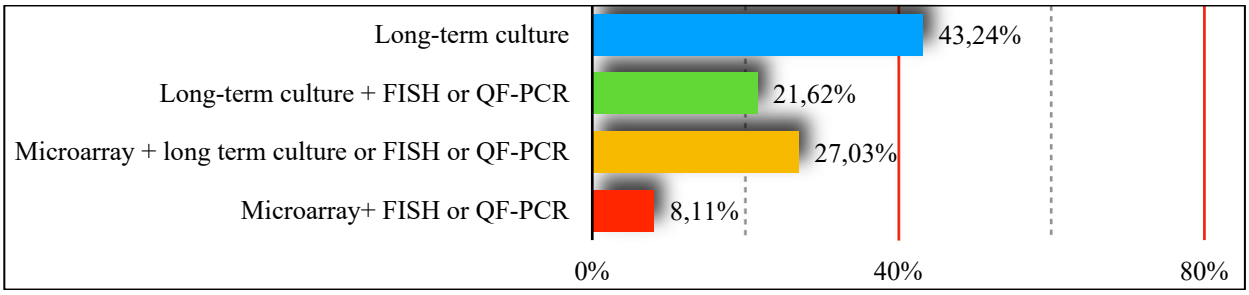
7. Per routine, do you combine the analysis on Cytotrophoblast cells with analysis on mesenchymal cells?



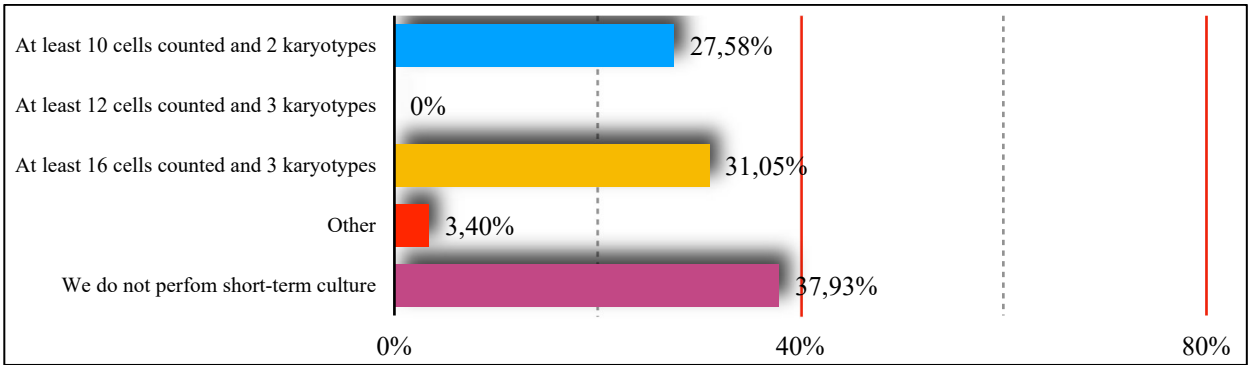
8. Which is the approach for fast analysis that you use in your laboratory? (you can select more than 1 option)



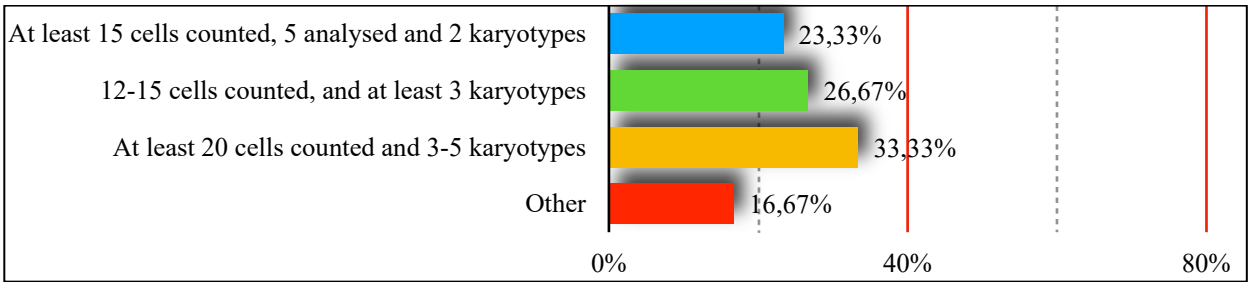
9. Which is the approach for long-term analysis that you use in your laboratory? (you can select more than 1 option)



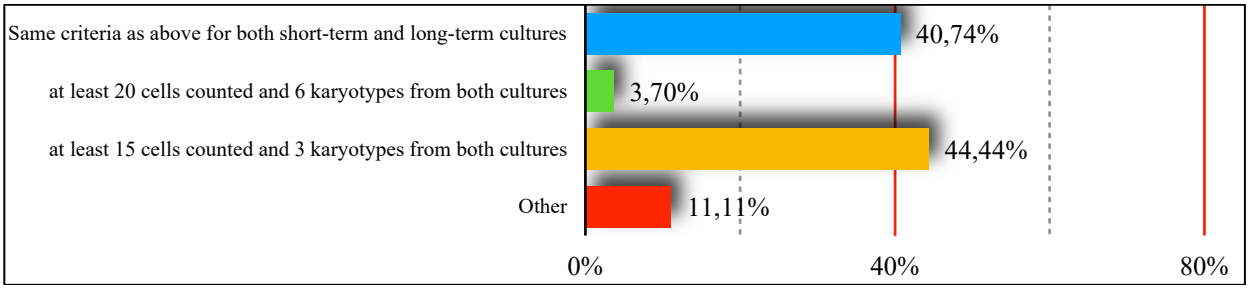
10. If you perform short-term culture (with FISH/QF-PCR), how many cells do you analyse?



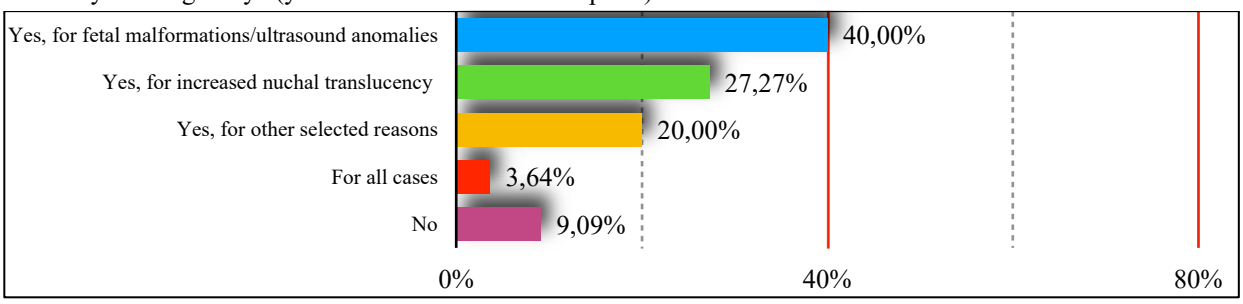
11. If performed long-term culture, how many cells do you analyse?



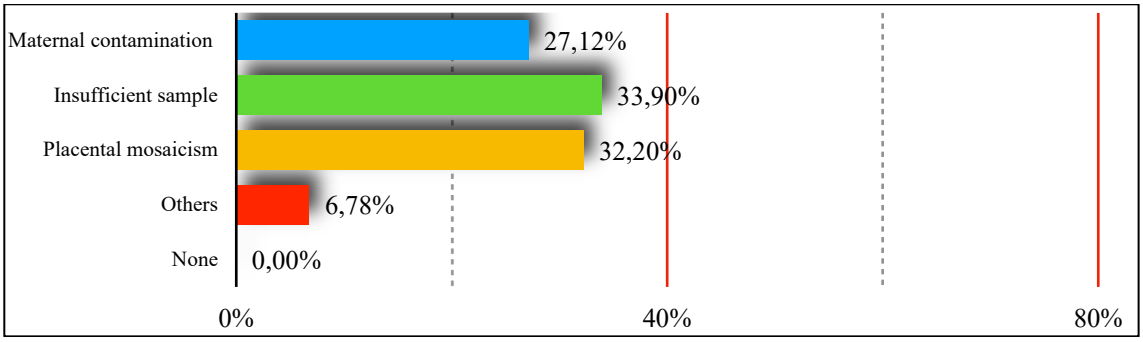
12. If performed long-term culture (after short culture or FISH or QF-PCR or Microarray), how many cells do you analyze?



13. Are you using array? (you can select more than 1 option)

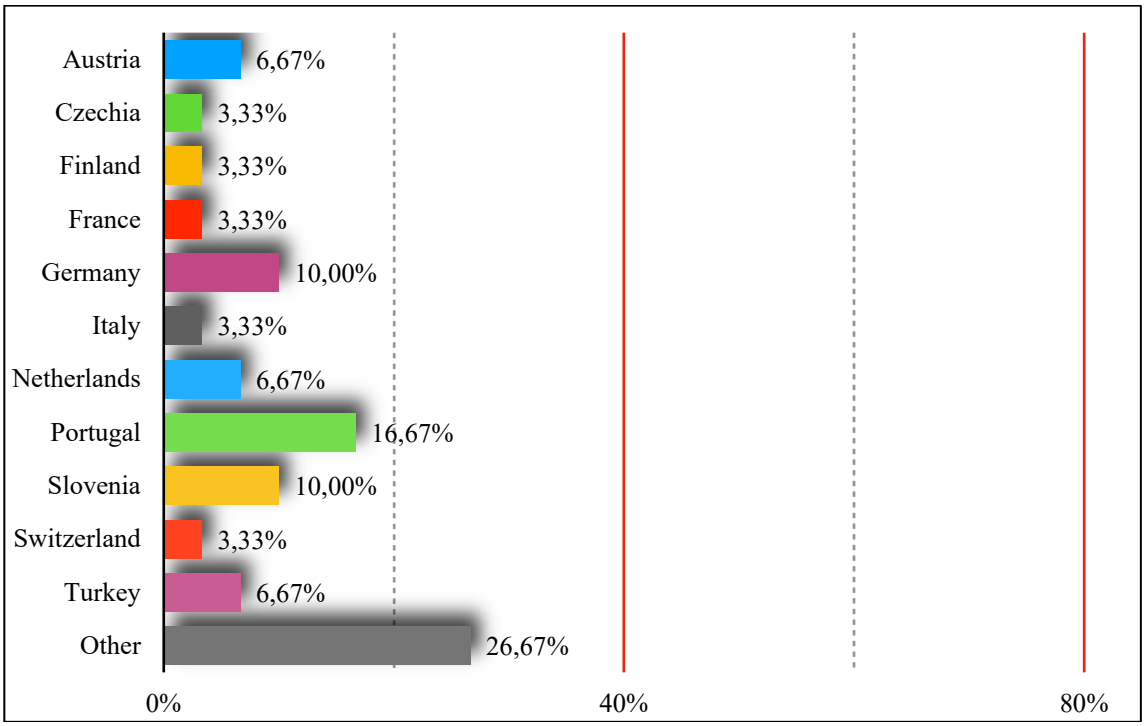


14. What do you consider to be the major problem(s) in chorionic villus sampling? (you can select more than 1 option)

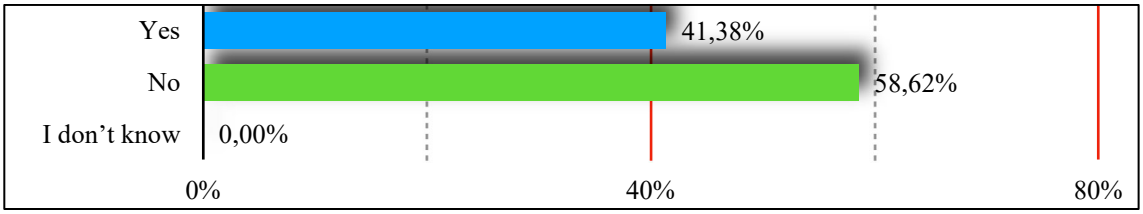


C) Guidelines

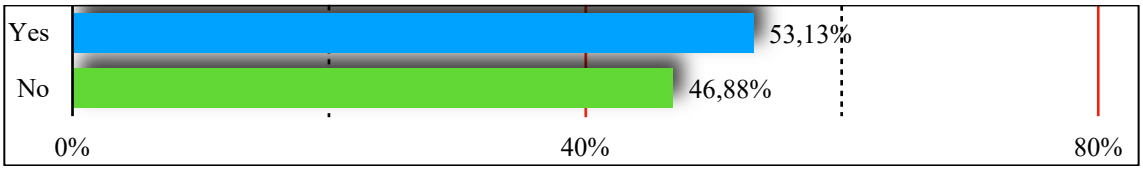
15. In which country do you work?



16. Do you have specific Guidelines for analysis of Chorionic Villi in your country?



17. Do you use the Guidelines from E.C.A.?



Conclusion

Despite being a very small study, we can try to draw some conclusions from this survey.

Concerning the questions related to the prenatal testing organization, we are aware that this part depends a lot on the center's expertise in performing the technique. However, as expected, most of the laboratories involved in prenatal diagnosis receive both amniotic fluid and CVS. While amniotic fluid is by far the main fetal sampling method, CVS represent around 1/3 of the prenatal samples for 58% of the laboratories. The usual time of collection is between 10-13 weeks, which is the correct timing to perform CVS. The main reasons for referral are an abnormal ultrasound finding followed by a positive biochemical screening, as it should be. Quite unexpectedly, almost 10% of cases are performed to confirm an abnormal NIPT result, where a control on amniotic fluid is a better option to make sure that the fetus is really affected. Maternal age referral reason continues to be present, although in a low percentage (5,95%). The quantity of CVS, when it can be measured, varies predominantly between 5 to 10mg, which is expected, but more than 10mg has also been observed.

Regarding issues related to the cytogenetic workflow for the CVS analysis, conventional cytogenetics remains the main technique, although here we have to consider different approaches. Only half of the responding laboratories do both the short and long-term analysis methods, and when the fast approach is applied the technique that predominated is RT-PCR, followed by short-term culture. Regarding the long-term analysis, the classical approach (conventional cytogenetics with FISH or RT-PCR) with or without microarray prevailed, although some colleagues only perform the long-term culture.

As for the number of cells analysed, in the two types of culture, it varied. For the short-term culture, two main strategies prevail, with either 10 or 16 analyzed cells while protocols seem to be more diverse for long-term karyotyping.

The array technique, as expected, is present in the cytogenetics laboratory, but as seen in the answer to question 9, only a minority of labs skip from conventional cytogenetic to microarray-only for mesenchyme analysis (8%) and even fewer do microarrays for all samples (3%).

Finally, national Guidelines for CVS analysis are available in 40% of the countries represented in the survey, while only 53% of laboratories claim they follow the E.C.A Guidelines in spite of the expected 60%. However, in general, for those who work with CVS all the answers fit these guidelines.

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Comments and suggestions may be sent to the authors

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Due to the pandemic, the 2021 course will be organized online. It will be held from March 1st to March 13th, 2021 with a schedule that is adapted to accommodate all time zones The lectures will be from 11am to 2pm Paris time (CET) with one extra hour of free discussion with the teachers on the topics of the day. Registration deadline is January 31st, 2021.

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 is €884. For payment by institutions and for more information, please contact the organizers.

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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.

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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.
- The students will have the opportunity to perform a test at the end of the course.

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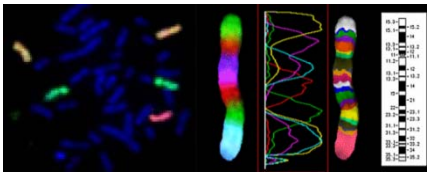

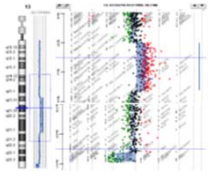

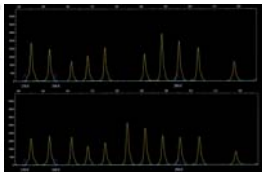

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