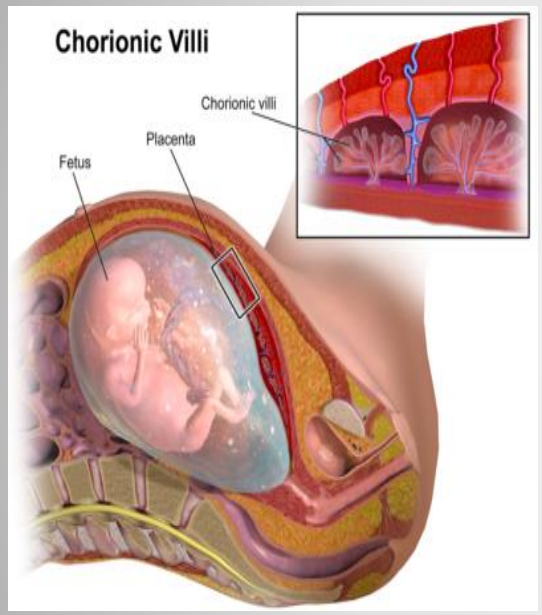
Technical Problems Solvings in Chromosome Study in Prenatal Diagnosis

Dr . Worapa Heepchantree

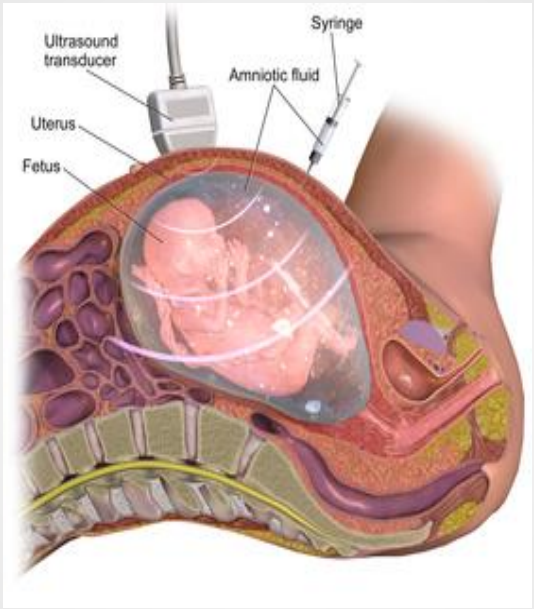
Prenatal Diagnosis

Chrorionic villi : CVS



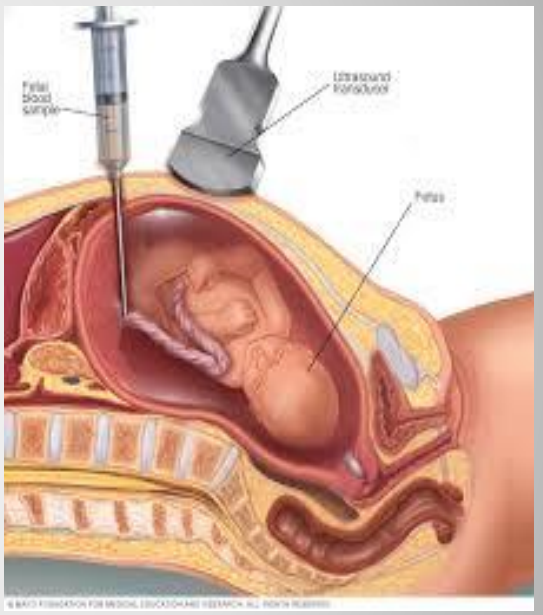
https://www.bornontario.ca/en/pso/results-and-next-steps/chorionic-villus-sampling.aspx

Amniotic fluid : AF



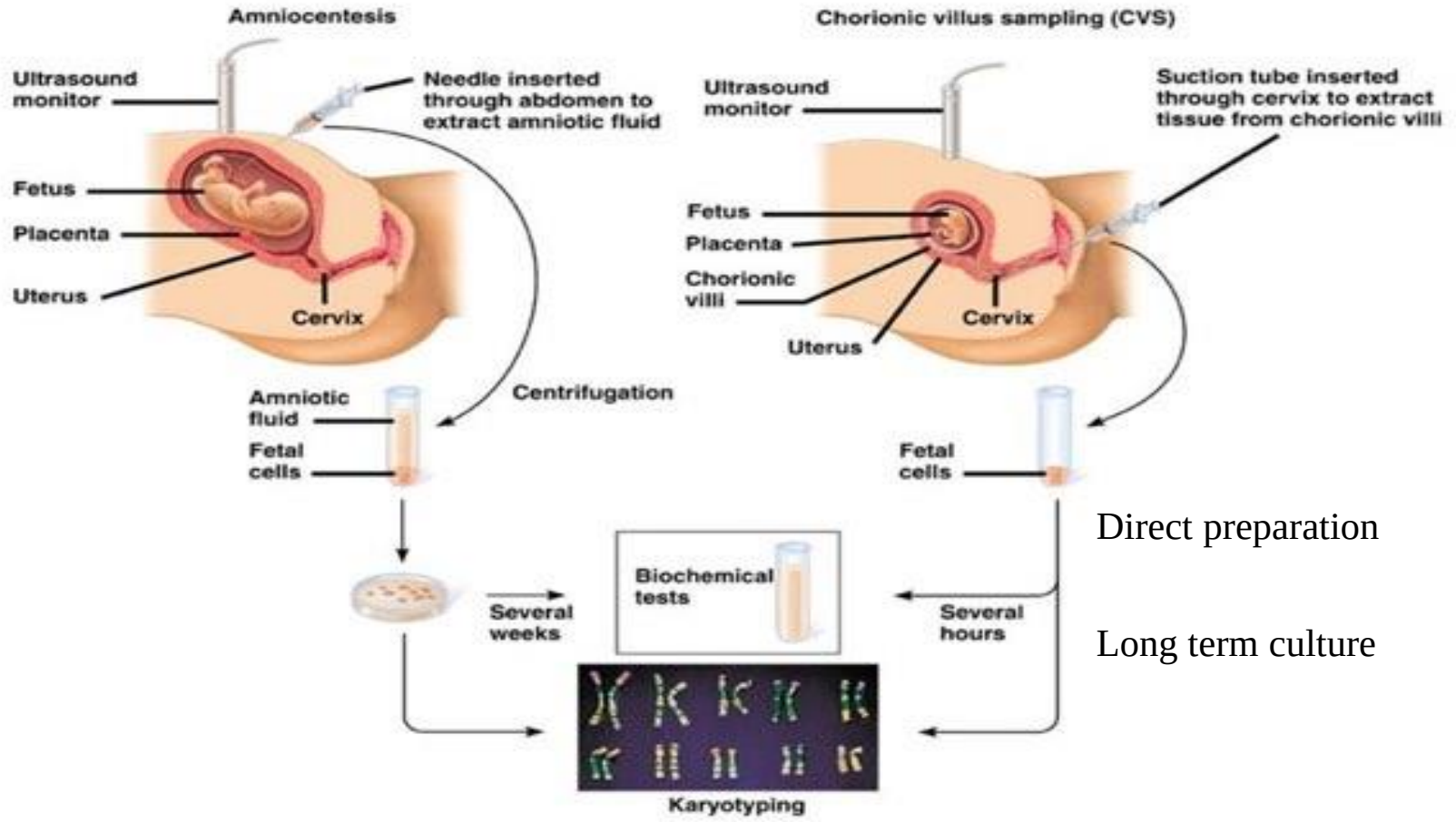
https://en.wikipedia.org/wiki/Amniocentesis

Umblical blood/Cord blood



https://www.mayoclinic.org/tests-procedures/percutaneous-umbilical-blood-sampling/about/pac-20393638

Amniocentesis & CVS



Direct preparation

Long term culture

https://www.qdosultrasound.com.au/pregnancy/cvs-amniocentesis-perth/

Culture initiation

Type of cells	AF/CVS*	Cord blood*
living cells	√	√
sterility	√	√
microbial inhibitor	penicillin-streptomycin	penicillin-streptomycin
mitotic stimulation	X	PHA
culture vessel	tissue culture flask	bottle/tube
growth medium	Chang /Bio-Amf`/Q3-21	RPMI+20% FCS

* Testing for maternal cell contamination

Culture maintenance

Type of cells	AF/CVS*	Cord blood*
media feeding	√	X
optimum pH	7.2-7.4	7.2-7.4
sterility	√	√
optimum temperature	37 ^{o}C	37 ^{o}C
optimum humidity	97 %	97 %
optimum time interval	10-14 days	72 hours

Cell harvest

- arrest division: colchicine, colcemid
- swell cell: Hypotonic solution 0.075M KCl
- fix cell: 3:1 (methanol:acetic acid)
- prepare slide
- banding/staining

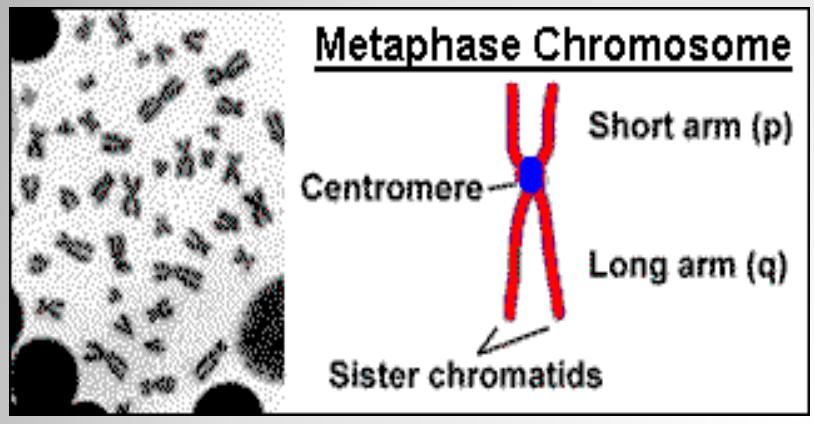
Chromosome banding/staining

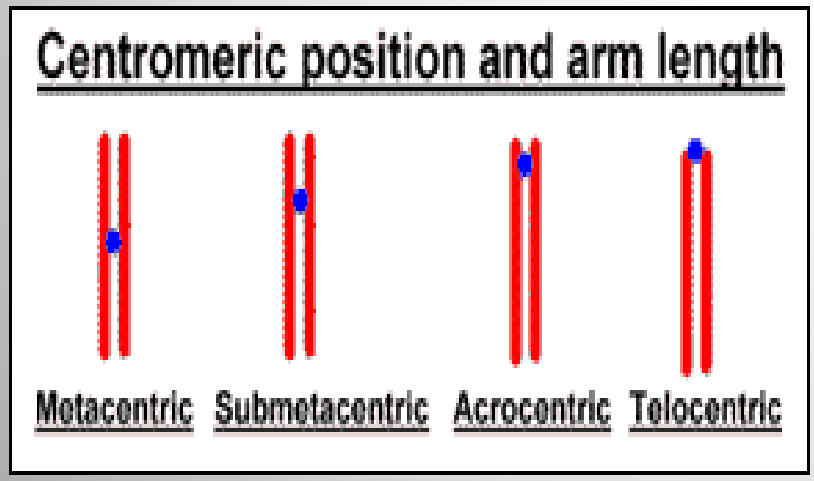
G-banding : G-band using Trypsin and Giemsa (GTG)

Q-banding : Q-band using Fluorescence microscopy and Quinacrine(QFQ)

C-banding : C-band using Barium hydroxide and Giemsa (CBG)

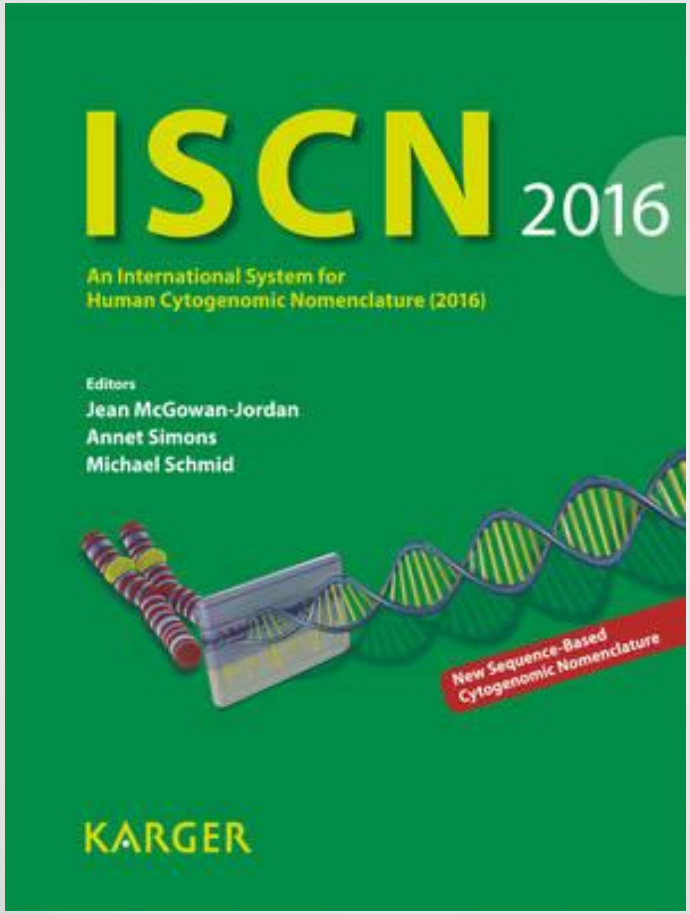
Identifying specific chromosomes





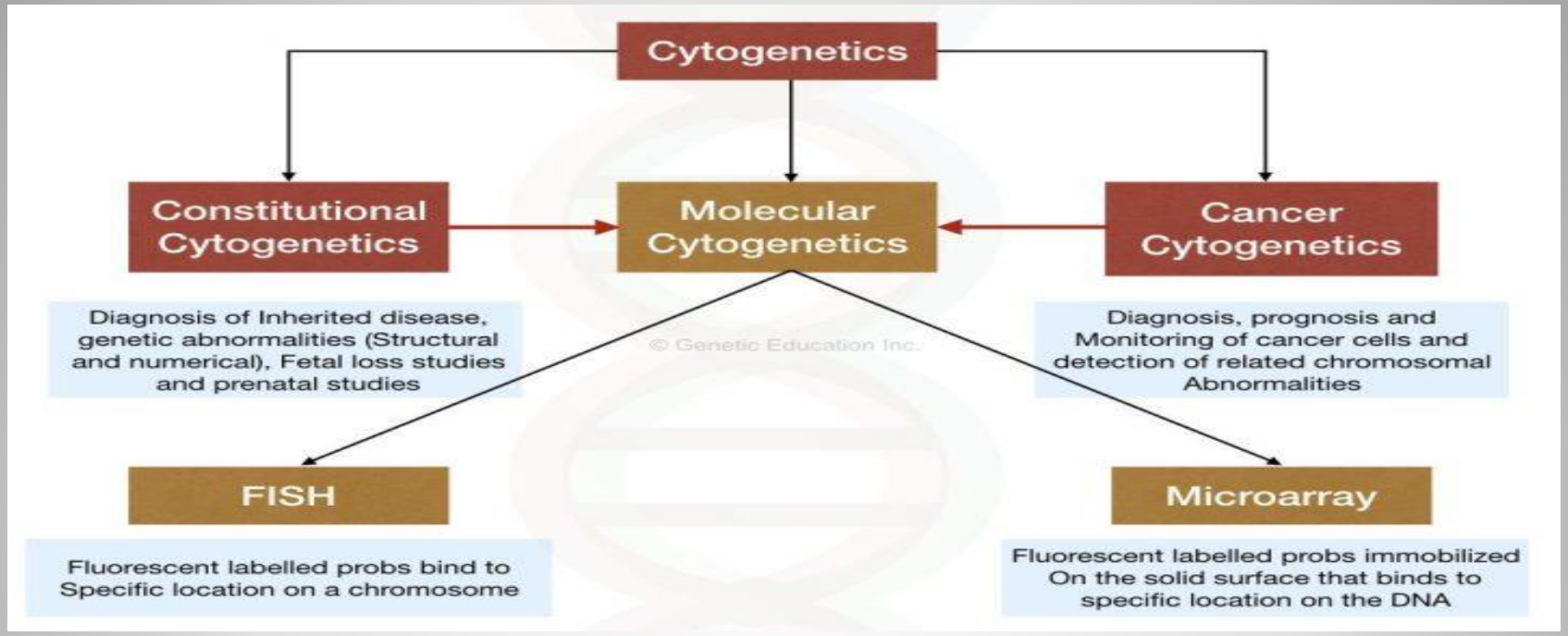
- Centromere position and arm ratios assist in identifying specific chromosomes
- Many chromosomes appear identical by these criteria.
- Identifcation revolutionised by dyes producing reproducible patterns of bands

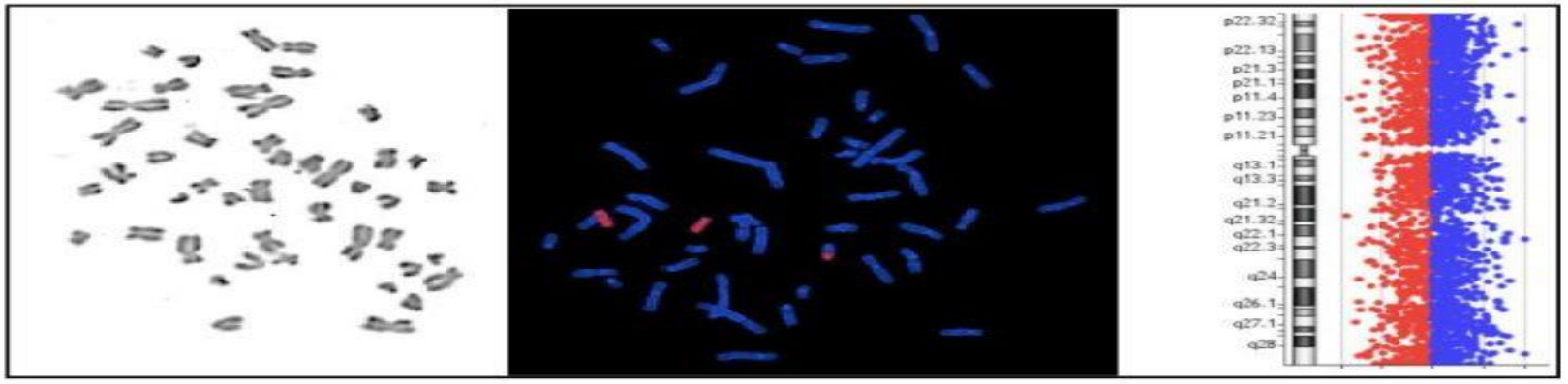
Analysis


ISCN 2016
An International System for
Human Cytogenomic Nomenclature (2016)
Editors
Jean McGowan-Jordan
Annet Simons
Michael Schmid
New Sequence-Based
Cytogenomic Nomenclature
KARGER

Discussion

I. Human
II. Policy
III. Technology





http://geneticeducation.co.in/a-brief-introduction-to-cytogenetics-karyotyping-fish-and-microarray/

Cytogenomic

Cytogenomic = Cytogenetic+omics

Array CGH

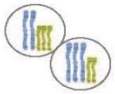
Exome sequencing

Genome sequencing (next generation sequencing)

others molecular genetics

to seek greater clarity in the analysis of chromosomal aberrations

Table 1: Genomic Technologies for Cytogenetics

	Parameter	FISH	Karyotyping	Arrays	NGS (large gene panel)	NGS (whole genome)
	Whole-Genome View	No	Yes	Yes	No	Yes
	Resolution	> 50 kb	> 5 Mb	< 1 kb	1 base pair	1 base pair
	Aneuploidy	Yes	Yes	Yes	Yes	Yes
	Unbalanced Translocation	Yes, if known	Yes, if large	Yes	Yes[a]	Yes
	Balanced Translocation or Inversion	Yes, if known	Yes	No	No	Yes
	Mosaicism	Yes	Yes	Yes, if 20% of cells present	Yes	Yes
	Polyploidy	Yes (indirect)	Yes	Yes (SNP arrays only)	Yes	Yes
	UPD	No	No	Yes (SNP arrays only)	Yes[b]	Yes
	Copy-Neutral LOH	No	No	Yes (SNP arrays only)	No	Yes
	SNVs	No	No	No	Yes	Yes
	Gene Fusions	Yes	No	Yes, if unbalanced No, if balanced	Yes	Yes

Using arrays and NGS to complement traditional methods, cytogeneticists can obtain a comprehensive view of genetic abnormalities, both large and small.[2]

a. Yes, if branch points are targeted.

b. Yes, if both parents are analyzed.

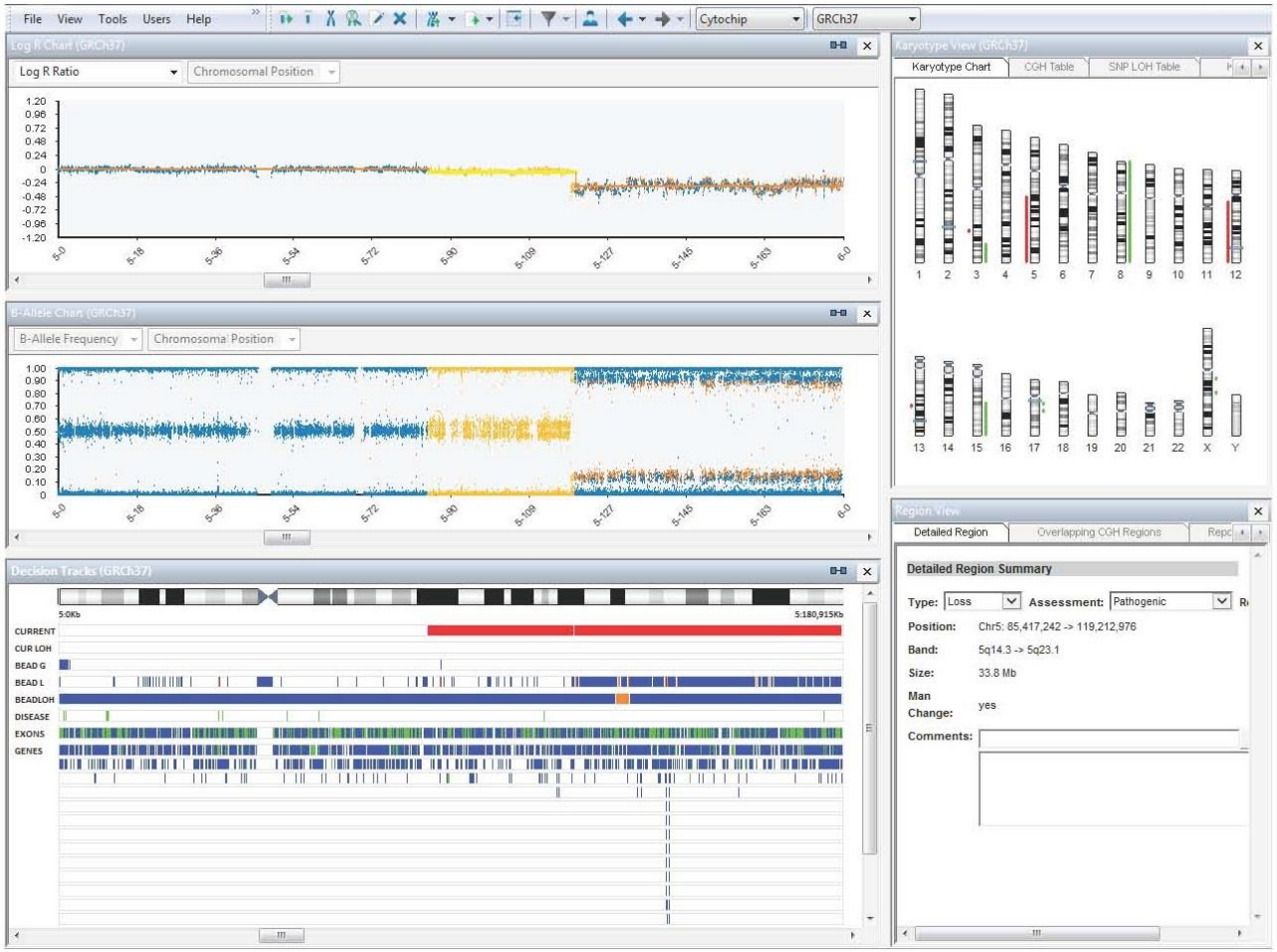
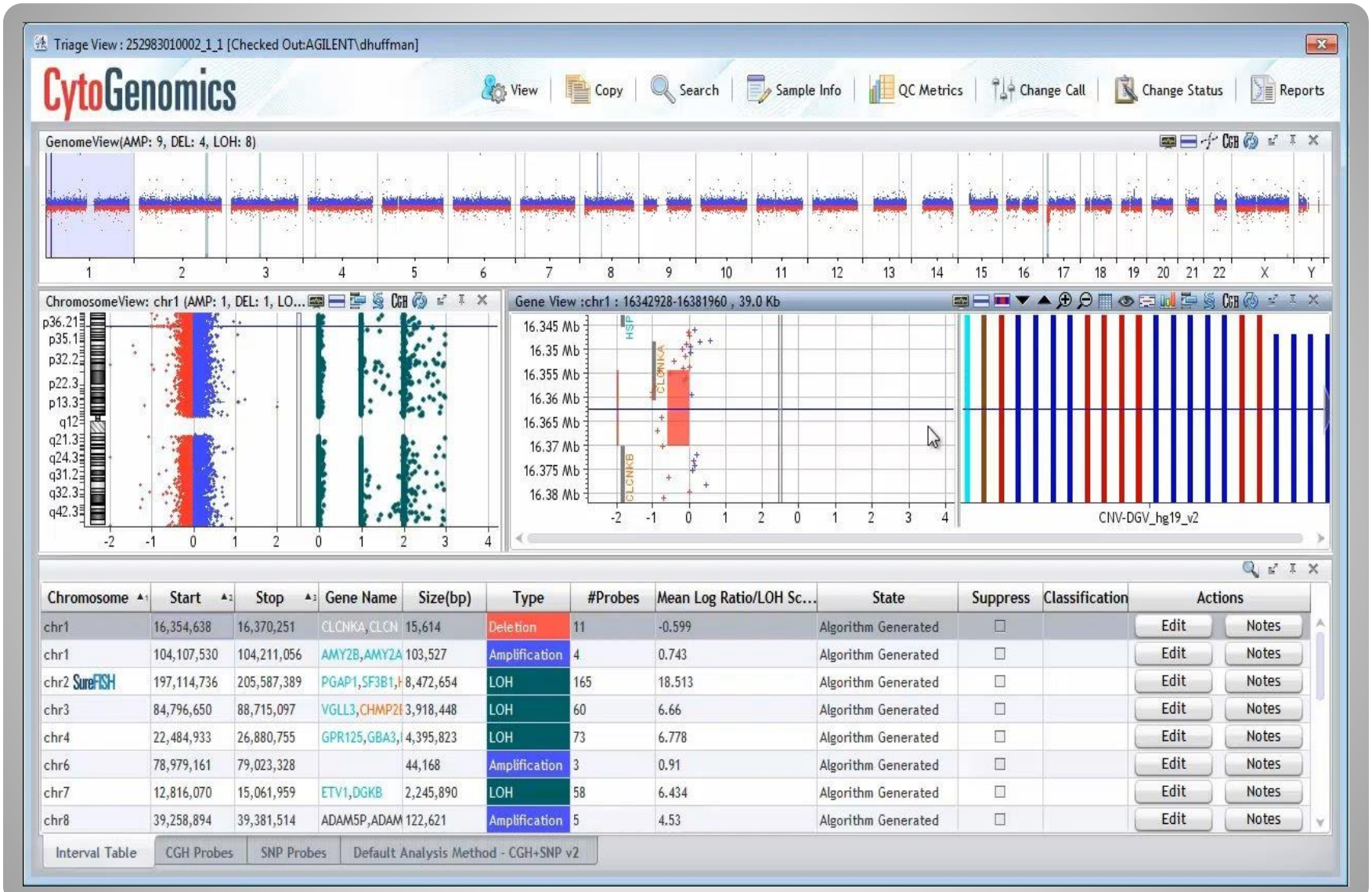


Figure 1: Genome-Wide View of Chromosomal Alterations—Horizontal view of the entire genome from BlueFuse® Multi Software showing mosaicism detection. By correlating the intensity information from the log R ratio (top plot) and the genotyping information from the B Allele Frequency Chart (middle plot), a mosaic deletion is evidenced on the long arm of chromosome 5. A region demonstrating low-level mosaic loss (highlighted yellow) lies adjacent to a region of high-level mosaic loss, which is indicated by the position of the heterozygous track on the B Allele Frequency Chart. The mosaic is unlikely to have been correctly characterized on an array CGH platform, which provides intensity information only. Data shown is from the Infinium CytoSNP-850K BeadChip.


Triage View : 252983010002_1_1 [Checked Out:AGILENT\dhuffman]
CytoGenomics
View
Copy
Search
Sample Info
QC Metrics
Change Call
Change Status
Reports
GenomeView(AMP: 9, DEL: 4, LOH: 8)
ChromosomeView: chr1 (AMP: 1, DEL: 1, LO...
Gene View :chr1 : 16342928-16381960 , 39.0 Kb
CNV-DGV_hg19_v2
Chromosome
Start
Stop
Gene Name
Size(bp)
Type
#Probes
Mean Log Ratio/LOH Sc...
State
Suppress
Classification
Actions
chr1 16,354,638 16,370,251 CLCNKA,CLCN 15,614 Deletion 11 -0.599 Algorithm Generated Edit Notes
chr1 104,107,530 104,211,056 AMY2B,AMY2A 103,527 Amplification 4 0.743 Algorithm Generated Edit Notes
chr2 197,114,736 205,587,389 PGAP1,SF3B1,H 8,472,654 LOH 165 18.513 Algorithm Generated Edit Notes
chr3 84,796,650 88,715,097 VGLL3,CHMP2E 3,918,448 LOH 60 6.66 Algorithm Generated Edit Notes
chr4 22,484,933 26,880,755 GPR125,GBA3, 4,395,823 LOH 73 6.778 Algorithm Generated Edit Notes
chr6 78,979,161 79,023,328 44,168 Amplification 3 0.91 Algorithm Generated Edit Notes
chr7 12,816,070 15,061,959 ETV1,DGKB 2,245,890 LOH 58 6.434 Algorithm Generated Edit Notes
chr8 39,258,894 39,381,514 ADAM5P,ADAM 122,621 Amplification 5 4.53 Algorithm Generated Edit Notes
Interval Table
CGH Probes
SNP Probes
Default Analysis Method - CGH+SNP v2

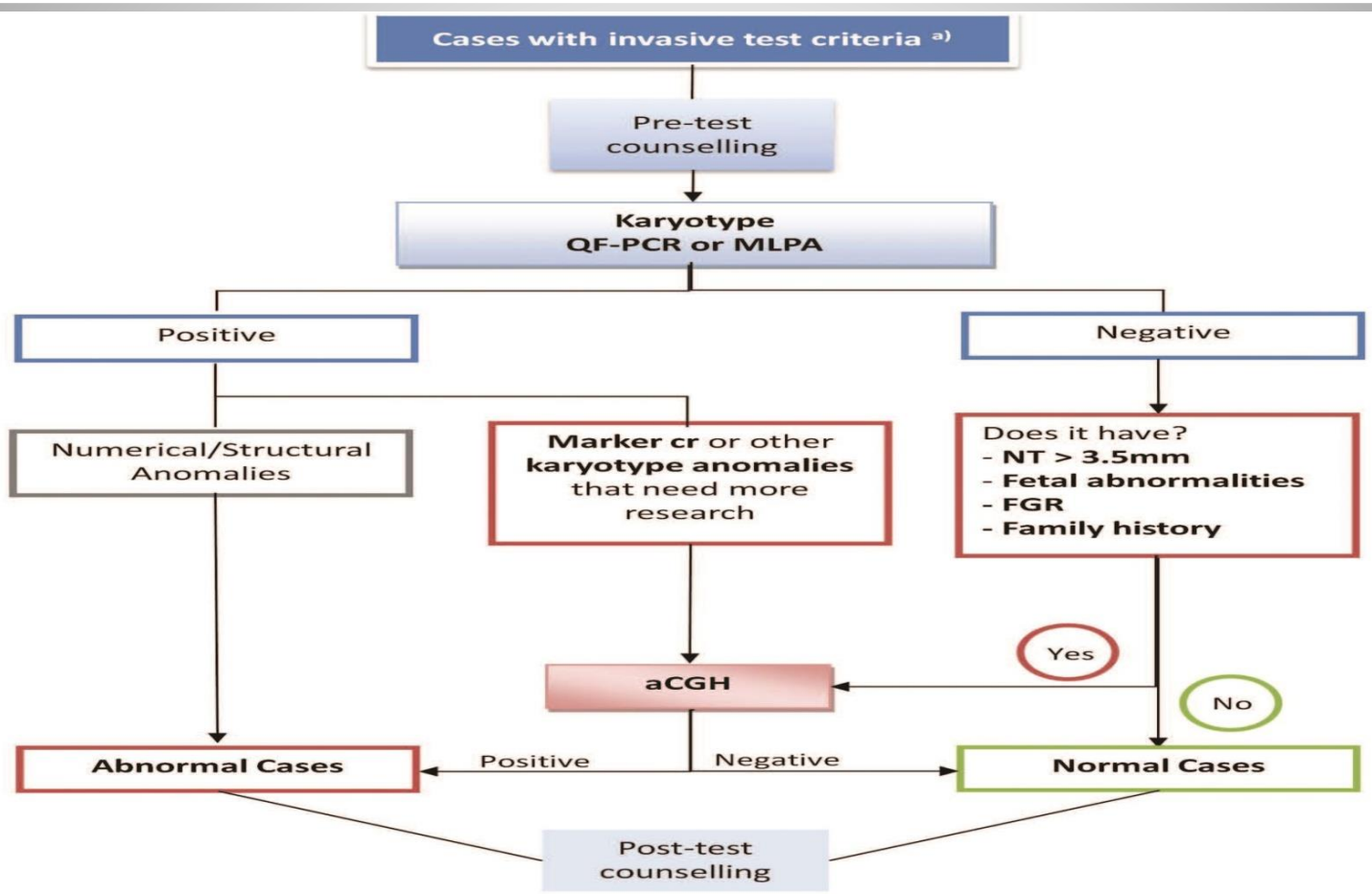


Figure 1. Algorithm of genetic prenatal diagnosis in Centro Hospitalar São João (CHSJ): tests and criteria. [a]Indications for invasive pregnancy test at CHSJ: positive combined first trimester screening, fetal abnormalities, nuchal translucency (NT) >3.5 mm (P99), early-onset fetal growth restriction (FGR), parents with balanced chromosomal rearrangements, family history of genetic disorder, and maternal anxiety.

European guidelines for constitutional cytogenomic analysis

Marisa Silva[1] · Nicole de Leeuw[2] · Kathy Mann[3] · Heleen Schuring-Blom[4] · Sian Morgan[5] · Daniela Giardino[6] · Katrina Rack[7] · Ros Hastings[7]

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Abstract

With advancing technology and the consequent shift towards an increasing application of molecular genetic techniques (e.g., microarrays, next-generation sequencing) with the potential for higher resolution in specific contexts, as well as the application of combined testing strategies for the diagnosis of chromosomal disorders, it is crucial that cytogenetic/cytogenomic services keep up to date with technology and have documents that provide guidance in this constantly evolving scenario. These new guidelines therefore aim to provide an updated, practical and easily available document that will enable genetic laboratories to operate within acceptable standards and to maintain a quality service.

Table 1 Methods used in cytogenomic analysis, their resolution and limitations

Method	Resolution	Limitations
Karyotyping	5–10 Mb	Cannot detect: small rearrangements below the resolution; nucleotide variants; mosaicism < 10%[a]; UPD[b]
FISH	~100 kb	Limited to the probes used (targeted analysis); Cannot detect: nucleotide variants; mosaicism < 10%[c]; UPD
Array-based techniques chromosomal microarray SNP-based array	~20–200 kb	Cannot detect: balanced rearrangement; mosaicism < 10%[d]; nucleotide variants; the nature of a structural aberration; independent cell lines; heterochromatic markers; triploidy (exception SNP array); UPD (exception SNP array)
CNV detection in whole-exome sequencing	100 bp (exonic regions) – ~150 kb (genome wide)[e]	Cannot detect: balanced rearrangement; mosaicism < 18%[f]; the nature of the structural aberration; independent clones/cell lines

[a]Hsu and Benn [7]; Hook [36]

[b]Uniparental disomy

[c]Wiktor and van Dyke [37]; Ballif et al. [38]; Mascarello et al. [39]

[d]Vermeesch et al. [23]; Ballif et al. [38]

[e]Pfundt et al. [32]

[f]Pagnamenta et al. [40]

Main clinical indications for prenatal diagnosis

- Abnormal foetal ultrasound;
- High-risk maternal serum screening/NIPT result indicating an increased risk of a chromosomally abnormal foetus;
- Parental chromosome rearrangement, mosaicism or previous aneuploidy;
- Previous livebirth/stillbirth with a chromosome abnormality;
- Possible foetal mosaicism detected by prior prenatal study;
- Familial monogenic disorder (i.e., CF, Noonan syndrome, etc).

Table 2 Recommended reporting times (calendar days) for 90% of the referrals

Prenatal aneuploidy testing by FISH /QF-PCR/MLPA	4 days
Amniotic fluid and CVS analysis on cultured or uncultured cells by karyotyping and/or genome-wide array analysis	14 days
Lymphocyte cultures	28 days
Products of conception/foetal skin (where pregnancy is not ongoing)	28 days
Urgent[a] lymphocyte, cord blood cultures	7 days
Postnatal array analysis	10 days (urgent) 28 days (other)

These apply in the absence of specific National Guidelines

[a]URGENT – referrals where the result will have immediate implications for patient management

Table 3 Minimum G-banding quality according to the reason for referral

Reason for referral	Minimum G-banding quality (QAS) [a]
Confirmation of aneuploidy	QAS 2[a] ⇔ < 300 bphs
Exclusion of known large structural rearrangements	QAS 3 ⇔ 300 bphs
Identification and exclusion of small expected structural rearrangements; routine prenatal specimen preparations	QAS 4 ⇔ 400 bphs[b]
Prenatal specimen abnormal ultrasound referrals (in the absence of array-based analysis)	QAS 5 ⇔ 550 bphs[b]
Routine postnatal specimen preparations	QAS 6 ⇔ 550 bphs[b]

bphs bands per haploid set

[a]QAS score – see ACC Professional guidelines for clinical cytogenetics v1.04 (2007; http://www.acgs.uk.com/media/765607/acc_general_bp_mar2007_1.04.pdf)

[b]SNP-based array or other molecular techniques/SNP-based array or other molecular techniques may be more applicable for some of these referral categories

Table 4 Minimum number of metaphases to be analysed according to tissue type

Sample	Referral/result	Minimum analysed[a]	Additional cells counted[b]
Prenatal	Routine	2 (2 cultures)[c]	0[d]
Postnatal	Routine	2[e]	0
Pre- and postnatal	Mosaicism exclusion or single-cell anomaly detection	2	28[f]

[a]Analysed (banded metaphases where every set of homologues without any crossovers are evaluated in their entirety). (In practice more metaphase are analysed to clear any crossovers – see section Karyotyping)

[b]Counted (metaphases where the number of centric chromosomes and/or the presence/absence of a specific cytogenetic feature is evaluated)

[c]QF-PCR and one culture in case of aneuploidy testing

[d]Extra cells may be counted to exclude mosaicism or a single-cell anomaly

[e]ACC Professional guidelines for clinical cytogenetics v1.04 (2007; http://www.acgs.uk.com/media/765607/acc_general_bp_mar2007_1.04.pdf) and Hastings et al. [1]

[f]Hsu and Benn [7] and Hook [36]

Table 5 Applications of FISH analysis

Type of FISH analysis	Applications of FISH analysis
Rapid prenatal FISH	High risk of chromosome aneuploidy or recurrent microdeletion (e.g., abnormal ultrasound); late gestation referral. Evaluation/characterisation of
Interphase FISH	Numerical abnormalities; Duplications; Deletions; Sex chromosome constitution; Mosaicism; Gene amplification.
Metaphase FISH	Marker chromosome; Unknown material attached to a chromosome; Rearranged chromosome(s); Suspected gain or loss of a chromosome segment; Mosaicism.

Table 6 Minimum criteria for analysing FISH results according with probe type

Probe type	Analysis	Additional comments
Locus-specific identifier (LSI) probes	5 metaphases	Score to confirm or exclude an abnormality (e.g., in case of suspected microdeletion syndrome or identification of a marker chromosome)
Multiprobe analysis	3 metaphases	Per probe. Scored to confirm a normal signal pattern. Confirmation is advisable for abnormal signal patterns if no control probe is present
Interphase analysis for aneuploidy testing	≥50 cells	For each probe set
Interphase analysis to detect mosaicism	≥100 cells	For each probe set

Prenatal diagnosis: the clinical usefulness of array comparative genomic hybridization

Marta Freitas, MD[a,b], Joel Pinto, BSc[b,c], Carla Ramalho, MD, PhD[c,d,e], Sofia Dória, PhD[b,c,*]

Abstract

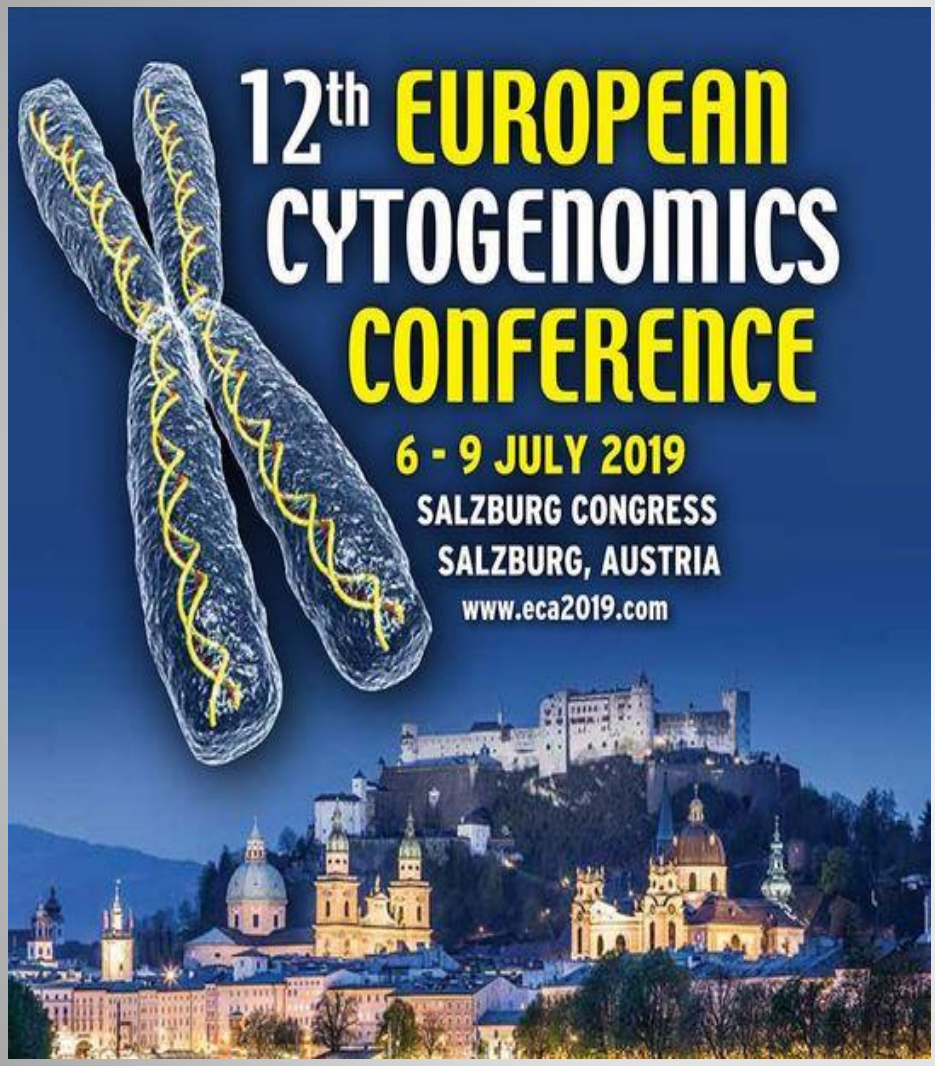
Background: Array comparative genomic hybridization (aCGH) has been replacing karyotype in neurodevelopment diseases or intellectual disability cases. Regarding prenatal diagnosis (PND) karyotyping is still the criterion standard technique; nevertheless, the application of aCGH in this field has been increasing dramatically and some groups recommended it as the first-tier prenatal genetic test in cases of fetal ultrasound abnormalities. Despite aCGH greater resolution, the detection of variants of unknown significance (VOUS) is not desirable, so it's need some reflexion before generalized application on PND.

Objective: The aim of this study was to analyze the prevalence and type of copy number variants (CNVs) detected in the 55 PND samples collected from pregnancies with indication to perform aCGH.

Methods: aCGH was performed using Agilent 4 × 180K microarrays and results were analyzed using CytoGenomics software.

Results and conclusion: Eight (14.5%) cases had pathogenic or likely pathogenic CNVs. VOUS were found in 21.8% of the cases, but this frequency could be minimized if only large CNVs above 1 million base pairs that are outside the clinically curated targeted regions were considered.

Keywords: array comparative genomic hybridization, copy number variation, karyotype, prenatal diagnosis, variants of unknown significance



Thank you for your attention