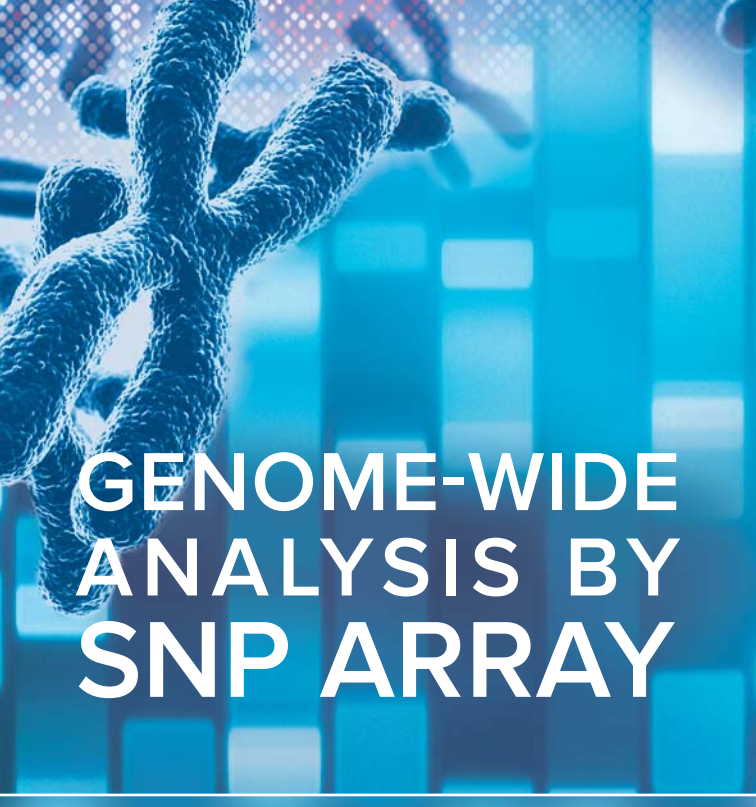


GENOME-WIDE ANALYSIS BY SNP ARRAY

SNP ARRAY IN THE DIAGNOSIS OF INTELLECTUAL DISABILITY AND CONGENITAL ABNORMALITIES

biomnis
SPECIALISED MEDICAL PATHOLOGY

Contents

Introduction

The emergence of DNA microarray (CGH Array/SNP Array) has revolutionised conventional cytogenetic diagnostics. This new technique analyses the whole genome with a higher resolution than that observed with classical karyotyping. Single nucleotide polymorphism (SNP) Array can detect and provide a detailed characterisation of the cryptic chromosomal anomalies implicated in mental retardation and congenital abnormality (CA), which are not detected by conventional methods. With the resolution advantage and due to the fact that this technology does not require a significant quantity of DNA, the scope of application of SNP Array is widened to include prenatal diagnostics. This review presents clinical cases that confirm the advantages of SNP Array in cytogenetic laboratory practice and defines the indications for the prescription of SNP Array testing.

Conventional and molecular cytogenetics (FISH)

1. Karyotyping

Karyotype analysis studies the number and structure of chromosomes (Figure 1). It gives an overall perception of the chromosome rearrangements within the whole human genome. Karyotyping is recommended when confronted with certain well-established clinical indications such as common aneuploidies (trisomy 21, 13 and 18), polymal-formative syndromes seen in newborn babies or during ultra sound examination, repetitive miscarriages, sterility, intellectual disability with dysmorphological features and intra-uterine growth retardation. The diagnosis of a chromosomal abnormality is crucial as it means that precise genetic counselling can be given. During pregnancy, the identification of an unbalanced foetal chromosomal abnormality is important for monitoring patient welfare.

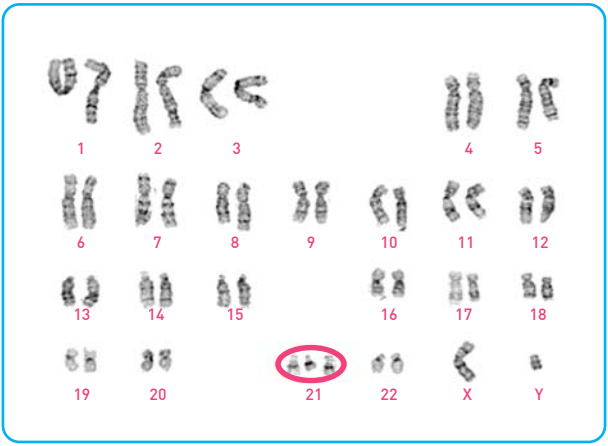


Figure 1. Karyotype of a child with Down's syndrome (47,XY,+21)

Karyotype limits

In certain cases, the pathologist can be confronted with chromosomal abnormalities that are difficult to characterise, or with cases where the clinical etiology strongly suggests the presence of a chromosomal abnormality but the karyotype is normal. In these situations, the karyotype reaches its limitation due to its low resolution. The most well known example is intellectual disability (ID). Despite the prevalence of ID (2-3%) and its impact on the individual and their family, karyotyping can only detect 5-10% of the patients suffering from ID (Figure 2). It could stem from chromosomal imbalances where the size is lower than the resolution of the karyotype (5-10 Mb).

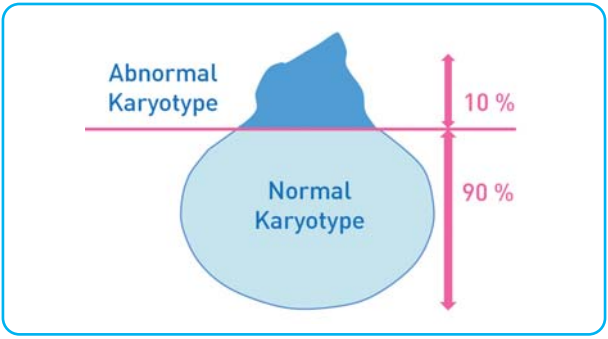


Figure 2. *The percentage of the detected anomalies by conventional cytogenetics for ID.*

2. Fluorescence *in situ* hybridisation (FISH): a targeted genome exploration.

FISH is based on the property of nucleic acids to follow the denaturation and hybridisation process under specific conditions of temperature, salinity and pH. A denatured probe (labelled single-stranded DNA) can specifically hybridise with its target sequence. Hybridised probes are then highlighted by immunodetection and detected using a fluorescent microscope. FISH can be performed on nuclei or metaphases with a resolution of 150 kilo-bases. Several types of probes (centromeric, painting, locus specific or telomeric; Figure 3) can be used to detect a chromosomal abnormality or confirm the presence of a known syndrome.

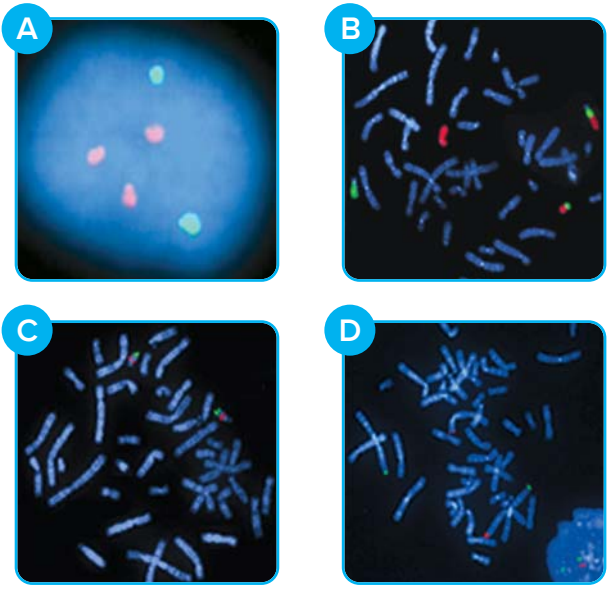


Figure 3. *FISH results showing hybridisation of centromeric probes on interphase nuclei* ***(A)****, painting* ***(B)****, locus specific* ***(C)*** *or telomeric probes on metaphase* ***(D)****.*

One of the common applications of FISH is in the diagnosis of chromosomal microrearrangements, which are usually specific microdeletions or microduplications syndromes including DiGeorge syndrome (22q11.2), Prader-Willi syndrome and

Angelman syndrome (15q11q13), Miller-Dieker (17q13.3) syndrome for the deletions, as well as Weideman-Beckwith syndrome (11p15) and Wolf-Hirschhorn syndrome (deletion 4p16) (Figure 4).

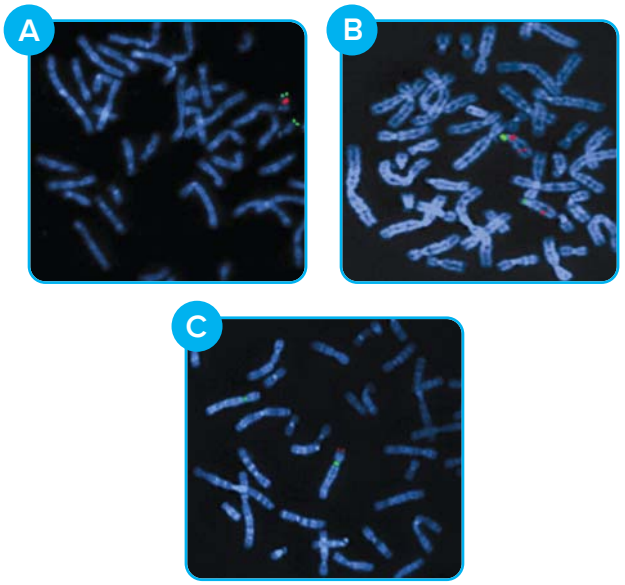


Figure 4. *FISH detection of Microdeletional syndromes.* ***(A)*** *DiGeorge Syndrome (deletion 22q11.2),* ***(B)*** *Prader-Willi syndrome and Angelman syndrome (deletion 15q11q13),* ***(C)*** *Wolf-Hirschhorn syndrome (deletion 4p16).*

FISH limits

The known microdeletional syndromes and micro-rearrangements of the terminal regions are only a small part of the pathologies that can be diagnosed by FISH. There remain numerous syndromes linking ID, CA and dysmorphia of unknown origin which could be caused by chromosomal microrear-rangements that are not diagnosed by FISH. This represents a limitation of the FISH technology. FISH does not examine the whole genome and can therefore study only the targeted regions with the help of specific probes.

Emerging technology: CGH/SNP Array for Genome-wide exploration

Human genome sequencing has allowed the understanding of microarrangement mechanisms involved in genesis of cryptic rearrangement implicated in ID and CA. These "genomic disorders" are the result of non-allelic homologous recombination (NAHR) between LCR sequences[2] "*Low Copy Repeat*". LCR makes up the molecular base of cryptic rearrangements whereby the direct consequence is DNA copy number variation. The deletions, insertions and duplications are qualified as copy number variations (CNVs) or copy number polymorphisms (CNPs).

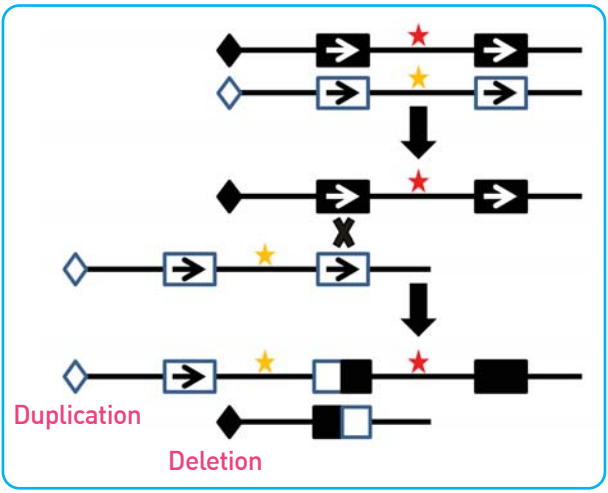


Diagram 1. *Schematic representation of non-allelic homologous recombination.*

A CNV is defined as a segment of DNA of 1000 bases or more which is present in a variable number of copies in comparison to standard DNA. CNVs can influence the gene expression by the deregulation of the genes or their regulator sequences, by the creation of fusion genes, or by directly altering the gene copy number.

CNVs can cause congenital diseases involving microduplications or microdeletions. All of the current scientific data reinforces the idea that the application of karyotyping or FISH remains insufficient for the diagnosis of the micro-rearrangements involved in ID and CA. Low karyotyping resolution (5-10 Mb) and the targeted analysis of FISH represent a significant restriction for ID and CA diagnosis. However, DNA microarrays have proved their utility in the diagnosis of ID and CA. They detect genomic disorders in 22.6% of patients reported as normal by karyotyping analysis[3].

In this review, we present the clinical interest of SNP Array use (HumanCytoSNP-12 BeadChip) in clinical genetic testing including ID, CA or infertility; in prenatal diagnosis and targeted identification of chromosomal markers not identified by karyotyping and the detection of the loss of heterozygosity (LOH) implicated in certain syndromes.

1. Comparative Genomic Hybridisation Array: CGH Array

CGH Array provides a pangenomic analysis of the human genome with a better resolution than that used in karyotyping analysis[4,5]. Currently, CGH Array is based on the competitive hybridisation of the DNA of the patient being tested (labelled by a green fluorochrome) and a normal DNA reference (labelled with a red fluorochrome) on a significant number of human DNA sequences (oligonucleotides spread across the whole human genome). After hybridisation, the fluorescence intensities of each oligonucleotide on the array is calculated using a scanner (Agilent®) and the fluorescence ratio is calculated to determine the copy number of each DNA marker tested. This calculated ratio detects the copy number variation (CNV) between the tested patient's DNA in comparison with the normal DNA reference. A CNV corresponding to a loss in genetic material (deletion) in the patients will be represented by a decreased fluorescent ratio whereas the CNVs corresponding to a gain in genetic material (duplication) show a raised fluorescent ratio (figure 5).

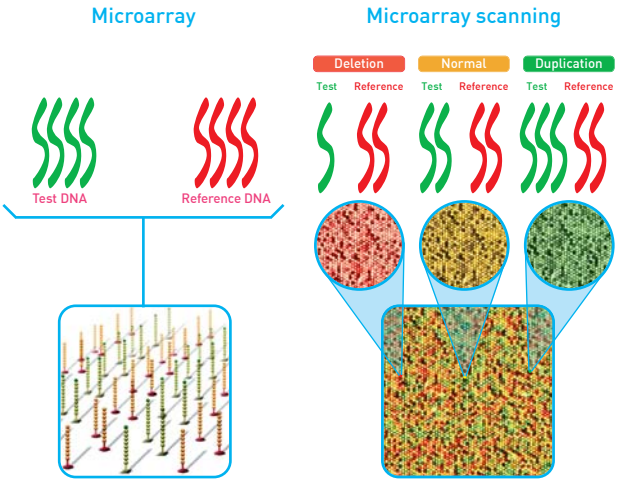


Figure 5. *Schematic representation of the CGH Array technology.*

2. Single Nucleotide Polymorphism Array: SNP Array

In comparison to CGH Array, SNP Array determines the CNV **and** the LOH (loss of genetic material of one of the two parents). In Biomnis, we use SNP Array technology (Illumina®). The HumanCyto-SNP-12 BeadChip used in our laboratory offers a whole-genome scanning panel. It includes 300 000 markers genome-wide tag SNP and markers targeting all regions of known cytogenetic disease. This includes dense coverage of approximately 250 genomic regions commonly screened by cytogenetic laboratories, including subtelomeric regions, peri-centromeric regions, sex chromosomes and targeted coverage in approximately 400 additionnal disease-related genes (www.illumina.com).

HumanCytoSNP-12 BeadChip detects CNVs and LOH relevant to many types of genomic variations including duplications, deletions, LOH and mosaicism. SNP Array offers CNV analysis using the intensity of the markers and the genotype using ***B allele frequency value (BAF).***

SNP Array is based on the whole genomic amplification, tagging and hybridisation on the Array slides. The BeadChips are then scanned using an iScan Reader (Illumina®) and the data analysis is performed using GenomeStudio and CaryoStudio (Illumina®). The BAF is the value between 0 and 1 and represents the proportion contribution of one SNP allele (B) to the total copy number. A BAF value of 0.5 indicates a heterozygous genotype (AB), whereas 0 and 1 indicates a homo-zygous genotype (AA, BB, respectively). In the case of a deletion, present in all cells, the deleted region will show homozygosity-bands at 0 and 1 (AA, BB genotypes) and loss of the BAF value at 0,5 (Loss of AB heterozygous genotype). Whereas, a region of single-copy-number gain, in all cells will, in addition to the two bands of homozygous SNPs at BAF = 0 (AAA) and BAF = 1 (BBB), also show two additional bands: one at BAF = 0,33 with SNPs

having genotype AAB and one at BAF = 0,67 with SNPs having genotype ABB (figure 6 and 7). The following resolutions are generally applied: Loss ≥ 150 Kb, gain ≥ 200 Kb and LOH ≥ 5 Mb.

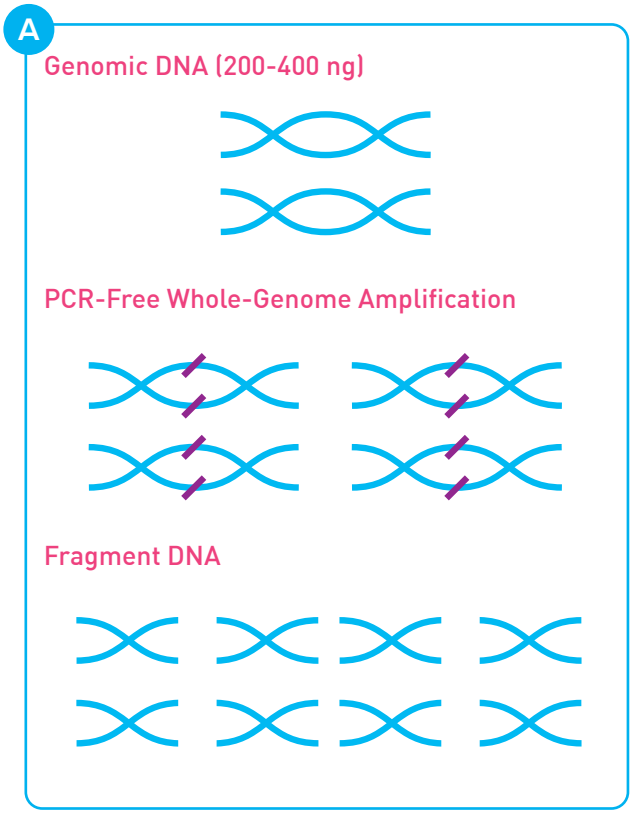


Figure 6 A. *Schematic representation of the SNP Array technique (Illumina®). DNA isolated from peripheral blood or amniotic fluid is amplified, fragmented and hybridised on the SNP Array.*

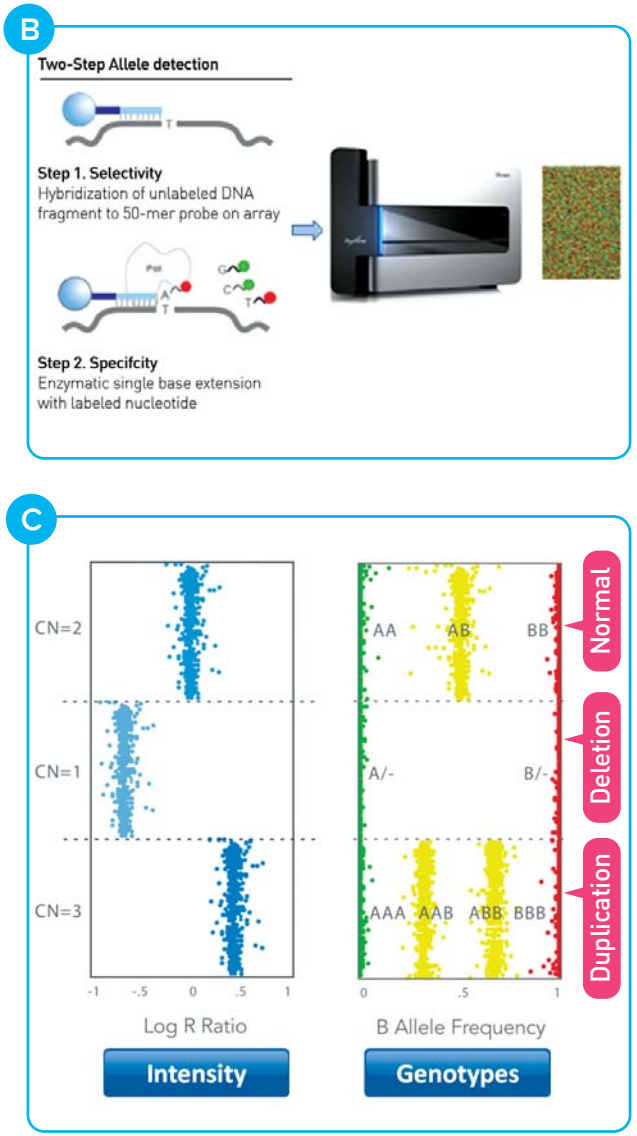


Figure 6 B. C. *The slides are scanned using iScan scanner (Illumina®) and the results are analysed using Genome Studio and Caryostudio Software. FISH or quantitative real-time PCR are used to confirm any abnormal findings either at the time of initial testing or upon receipt of parental samples, depending on the abnormality, while methylation-specific.*

Detection of a complex genomic changes in patient with ID

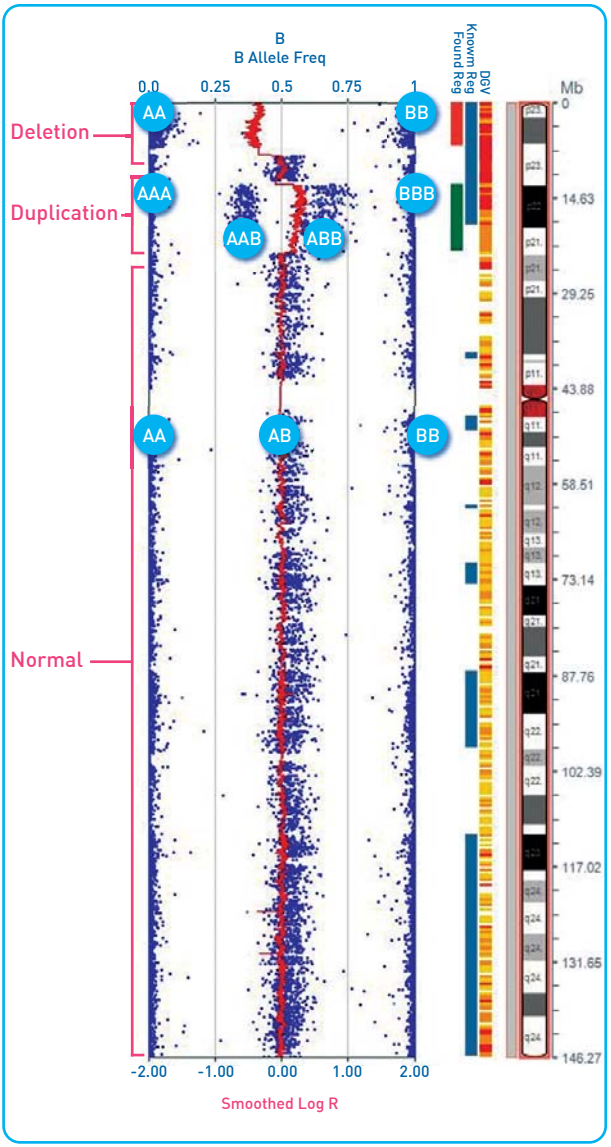


Figure 7. SNP Array results using HumanCytoSNP-12 BeadChip (Illumina®) showing complex genomic changes on chromosome 8p. [arr8p23.3.p23.1(221411-6914226)x1,8p23.1.p21.3(12583059-22995348)x3]

SNP Array validation and interpretation

UCSC (University of California, Santa Cruz) buit Hg19 is generally used to analyse data. Copy-number-variation (CNVs) are systematically checked in the public databases DGV (Database of Genomic Variants), Chop database and literature. Other databases are also used such as DECIPHER (Database of Chromosomal Imbalance and Phenotype in Humans using Ensemble Resources) and OMIM (Online Mendelian Inheritance in Man). The final results follow the International Standards for Cytogenomic Array Consortium nomenclature (ISCA 2009).

1. Pathological CNVs

The most important criterion to classify CNVs as pathological is its association with a known abnormal phenotype. CNVs with a direct association to an abnormal phenotype or known syndrome are assumed to be pathological. CNVs associated with a known increasing risk of an abnormal phenotype is also assumed to be pathological.

2. CNVs assumed as Polymorphisms

Parental DNA, when available, is tested by another technique such as FISH or Real time PCR, to identify inherited CNVs. Rare CNVs associated with an increased risk or abnormal phenotype, inherited from a healthy parent, is assumed to be benign. If a similar CNV was found in more than 3 individuals, the CNV is qualified as a polymorphism.

3. Unclassified LOH & CNVs

LOH is of unknown clinical relevance, except in cases of uniparental disomy of inherited regions. De *novo* CNVs or LOH of unknown (absence of disease-related gene) or uncertain clinical importance are defined as unclassified variants.

Clinical cases resolved by SNP Array

1. Application of SNP Array in the diagnosis of intellectual disability

- **Case 1: Detection of the Wolf-Hirschhorn syndrome.**

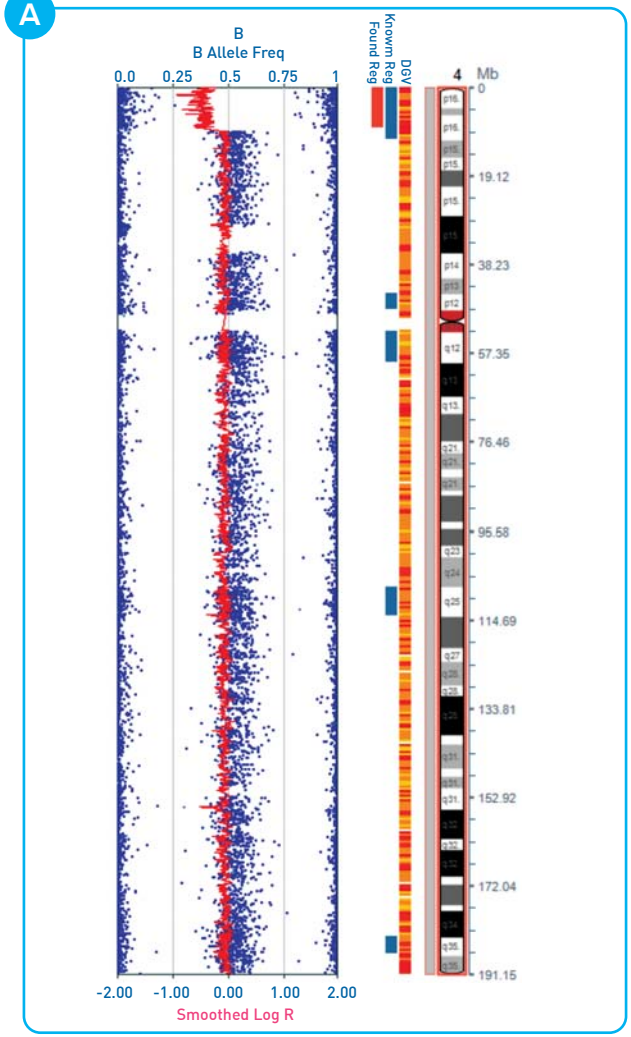


Figure 8 A. *SNP Array results of an infant, with intellectual disability. This result shows the presence of a deletion 4p16.3 of 8,7 Mb, [arr4p16.3p16.2p16.1(38,283-8,757,013)x1-8.7Mb] responsible for the Wolf-Hirschhorn syndrome.*

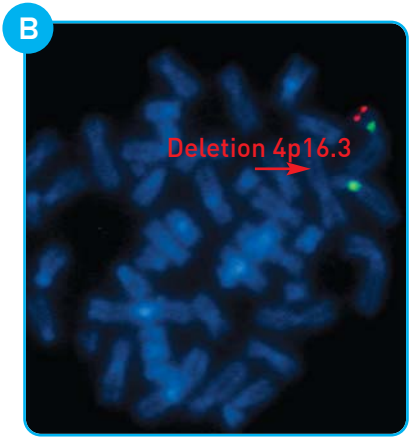


Figure 8 B. *FISH results confirming the presence of the Wolf-Hirschhorn syndrome (Red spot) on the chromosome 4 (Green spots).*

Wolf-Hirschhorn syndrome is the result of a chromosomal deletion at 4p16. This syndrome is characterised by growth retardation, muscle hypotonia and a developmental retardation with mental retardation.

Case 2: Detection of Prader-Willi and Angelman syndrome

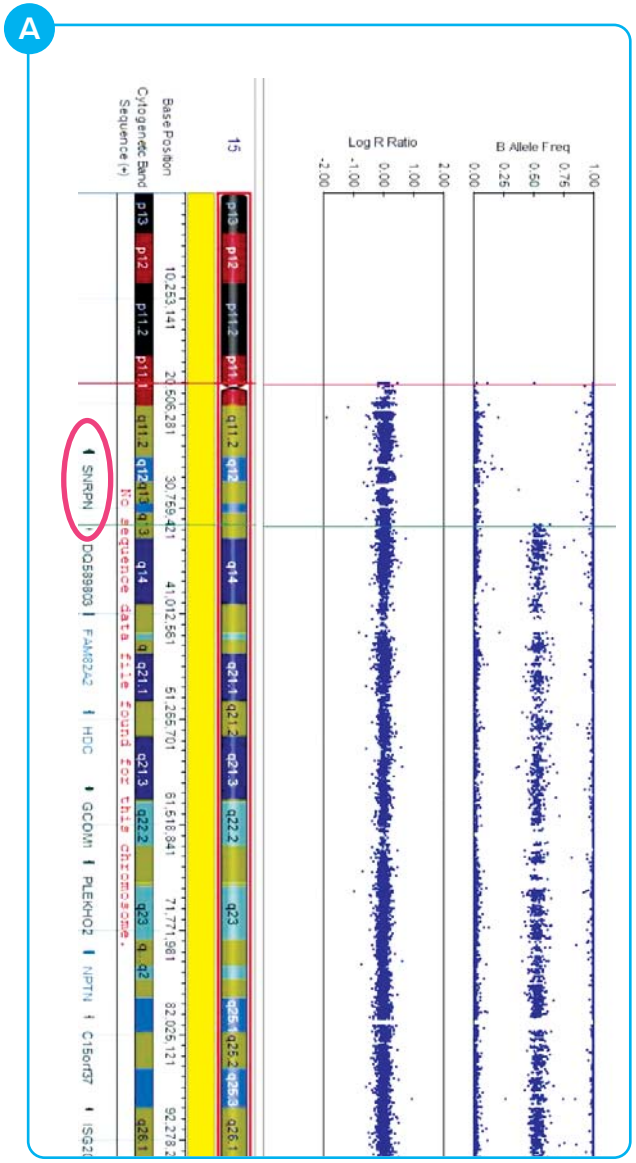


Figure 9 A. SNP Arrays results shows the presence of LOH on chromosome 15q11.2 harbouring the Prader-Willi and Angelman syndrome region.

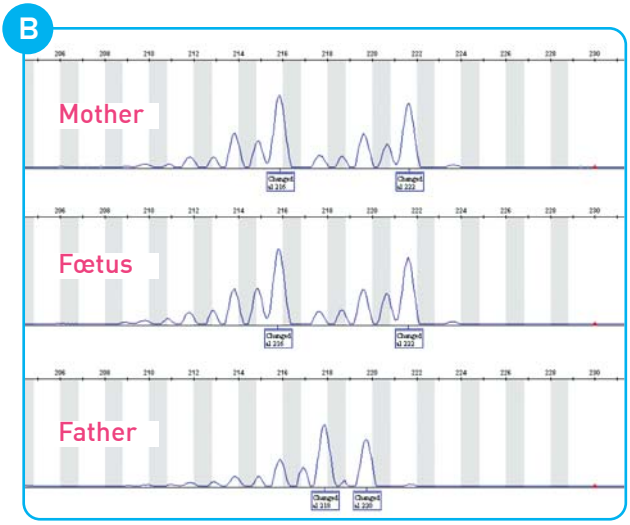


Figure 9 B. *Confirmation of PWS by PCR and fragment analysis using STR markers specific for chromosome 15.*

Prader-Willi (PWS) is a genetic disorder involving several genes on chromosome 15q11-13. This chromosomal region is influenced by "imprinting" which refers to expression of genes from one parent's chromosome with silencing of the other parent's chromosome. In the case of PWS, the paternal region is active, so that deletion or failure of inheritance of the paternal region causes the syndrome. PWS is characterised by mental retardation, hypogonadism, hypotonia, obesity, characteristic facial appearance. PWS is a result of paternal 15q deletion or maternal 15q uniparental disomy.

2. Application of SNP Array in the diagnosis of infertility

Case 3: Detection of genomic changes in patient with premature ovarian failure presenting a normal Karyotype

Patients with premature ovarian failure (POF) show partial deletions on chromosome X and X-autosome translocations. Seventy per cent of the deletions in the terminal end of chromosome X are responsible for POF[6]. The critical regions are located between Xq13.3 and Xq26-q27 containing POF1 (Xq21.3-q27) and POF2 (Xq13.3-q21.1), necessary for ovarian development. POF is also linked to a FMR-1 gene permutation involved in fragile X syndrome familial transmission. Studies have shown that 20% of permutated women have POF[7,8]. In a routine laboratory practice, the translocations of chromosome X and the screening for the FMR-1 gene is systematically performed for patients with POF. However, the karyotype analysis, due to its low resolution, gives normal results in some cases presenting cryptic chromosomal abnormalities (Figure 10).

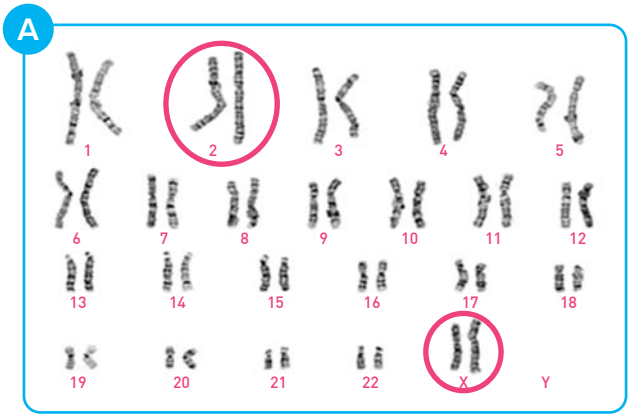


Figure 10 A. *Karyotype results showing normal karyotype 46,XX in a woman with Premature Ovarian Failure.*

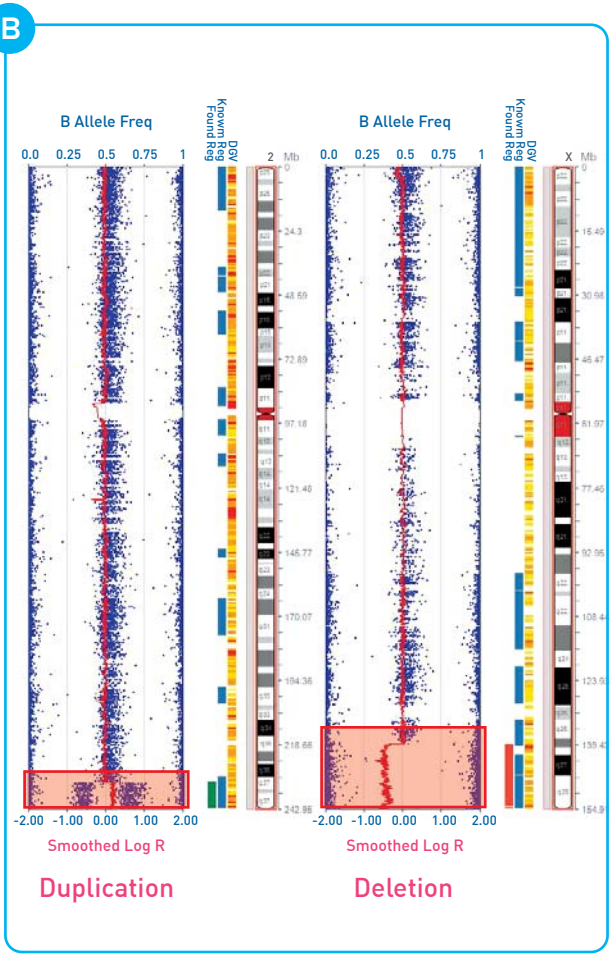


Figure 10 B. *SNP Array results of the same patient with POF showing the presence of duplication on chromosome 2 and a deletion on chromosome X. This result was confirmed by FISH (data not shown).*

3. Application of SNP Array for prenatal diagnosis

Case 4: Detection of genomic changes in fetus with ultrasound findings

Prenatal diagnosis requires fast and sensitive tests to manage abnormal pregnancies. Prenatal testing is commonly performed using karyotyping and FISH analysis. In certain cases, the pathologist is confronted with ultrasound results which strongly suggest chromosomal disorders (polymalformative signs, growth retardation, nuchal translucency, etc.), whereas the karyotype is normal. This is certainly due to the low resolution of the karyotype. In these cases, the interest of detecting these genomic changes by CGH Array or SNP Array becomes primordial as these techniques provide a pangenomic analysis with a better resolution[9-17]. The low quantity of DNA (50 ng) necessary to perform a SNP Array (without cell culture of amniotic fluid), the better resolution of the arrays in comparison to a karyotype, the simultaneous detection of CNVs as well as LOH and polyploidy represent additional arguments for the application of the SNP Arrays to prenatal diagnosis.

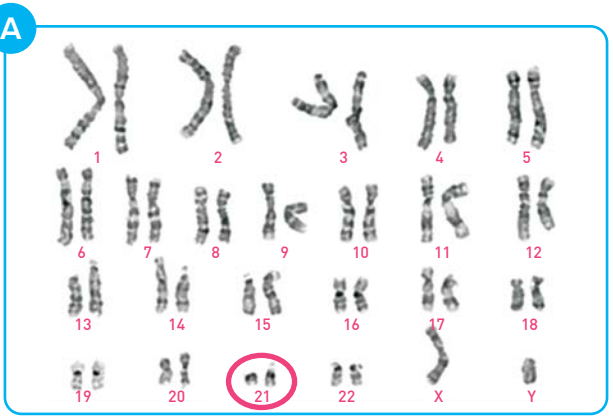


Figure 11 A. *Prenatal diagnosis of a fœtus with a karyotype suspecting an anomaly of chromosome 21.*

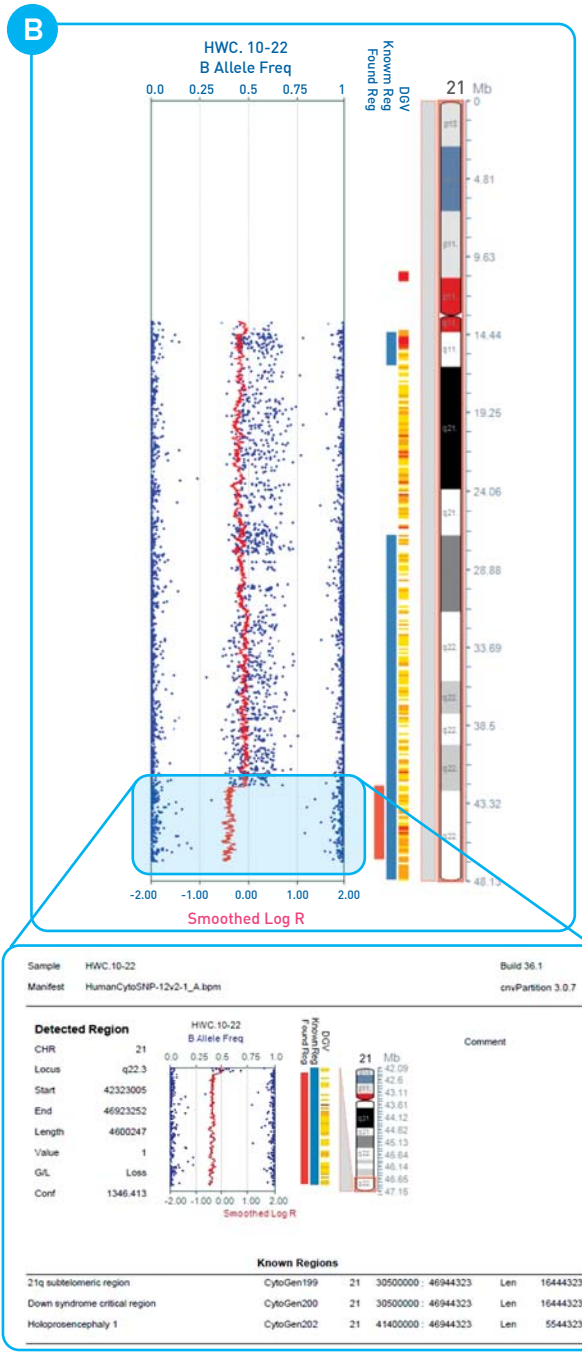


Figure 11 B. *The SNP Array results confirm the presence of a deletion in chromosome 21q of 4.6 Mb [arr21q22.3(42323005-46923252)x1] associated with holoprosencephaly phenotype.*

4. Targeted applications of the SNP Array

Case 5: "Balanced" rearrangements with an abnormal phenotype

Certain patients are carriers of translocations, insertions and inversions etc.), with no loss or gain in genetic material appearing on the standard karyotype. The same patients when analysed by SNP Array show the presence of duplication or deletion in the initial rearrangement, not detected by the karyotype. This is important when the karyotyping of the fœtus shows a seemingly balanced de *novo* translocation. Figure 12 shows a case of a fœtus with cryptic deletion, detected only by SNP Array, following an inversion of chromosome 12 resulting in severe mental retardation, delayed development, small head and craniofacial abnormalities.

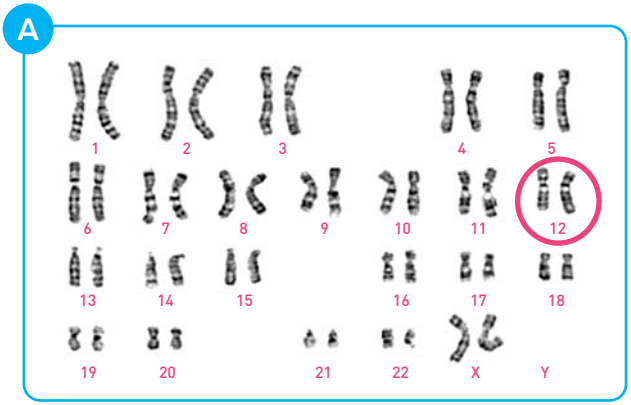


Figure 12 A. *Prenatal diagnostic showing an inversion on chromosome 12 (46,XX,inv(12)(p13.1q24.1).*

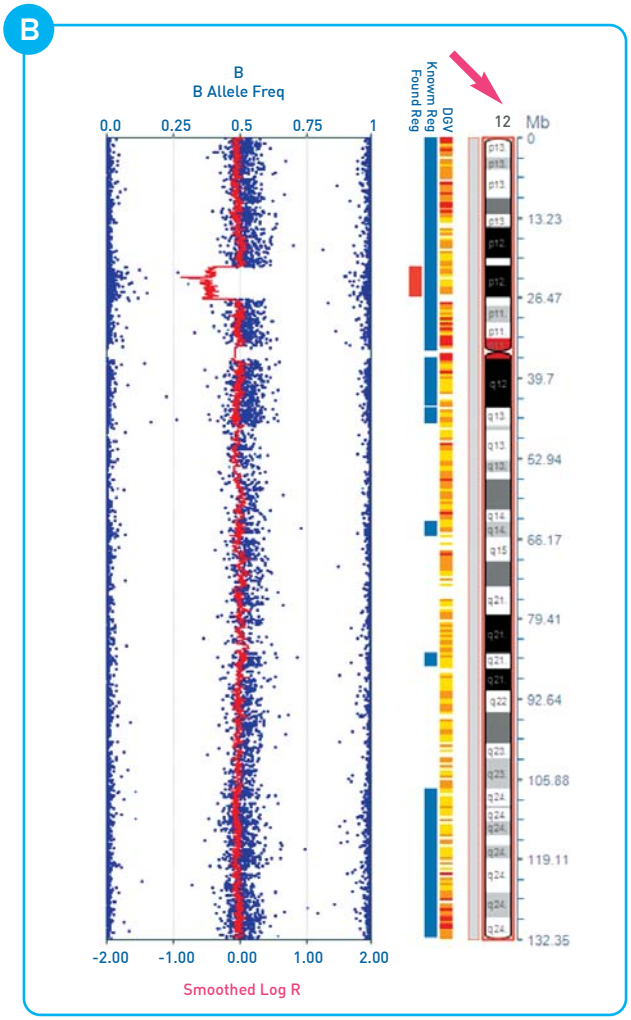


Figure 12 B. *SNP Array results of the same sample showing a microdeletion of 5.2 Mb at the break point of the inversion on chromosome 12 [arr12p12.1p11.23(21416873-26670081)x1].*

5. Detection of Supernumerary Chromosomal Markers (SCMs)

Case 6: Prenatal identification of chromosome marker

SCMs are defined as additional chromosomal of complex or abnormal structure. SCMs can be detected by karyotyping but their origin is usually difficult to identify. The structure of the SCMs is variable: derivatives (der), inversion duplication (inv dup), ring (r), isochromosome (i), minute chromosomes (min). SCMs are correlated with known clinical syndromes (Pallister-Killian syndrome and Cat-eye syndrome). Within the scope of prenatal investigations, the frequency of SCMs is estimated at 0.075%. They can be suspected during the ultrasound and/or when the mother's age is relatively old. In any case, it remains important to identify the origin and the structure of the SCMs and the presence or absence of euchromatin.

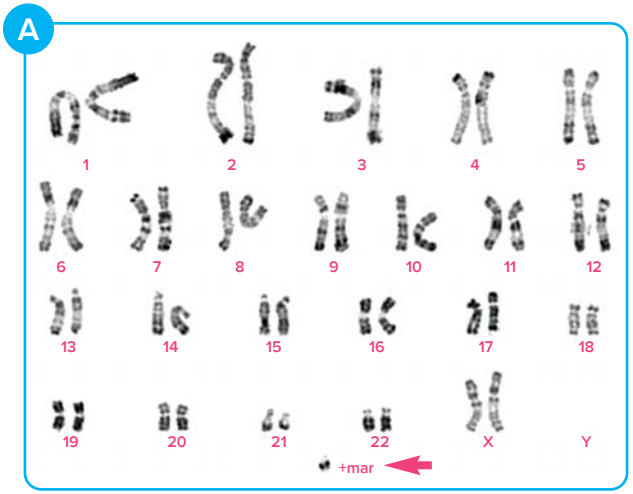


Figure 13 A. Prenatal karyotyping with a non identified marker.

B

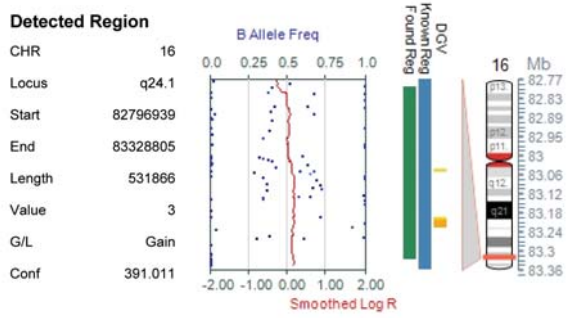


Figure 13 B. *The application of the SNP Array technology confirmed the origin of the marker. This marker is a result of a duplication of the chromosome 16q24.*

C

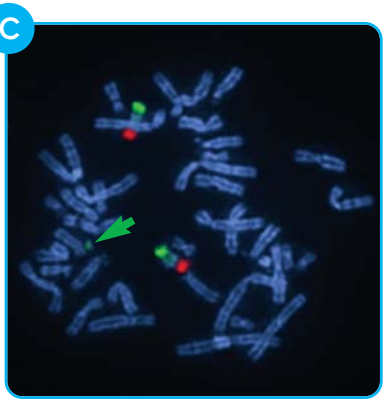


Figure 13 C. *This data was confirmed by FISH. Mos46,XX[20]/47,XX,+mar.arr 16q24(82796939-83328805)x3*

6. Benefits of SNP array in Products of Conception (POC)

Case 7: First trimester miscarriage caused by fetal triploïdy

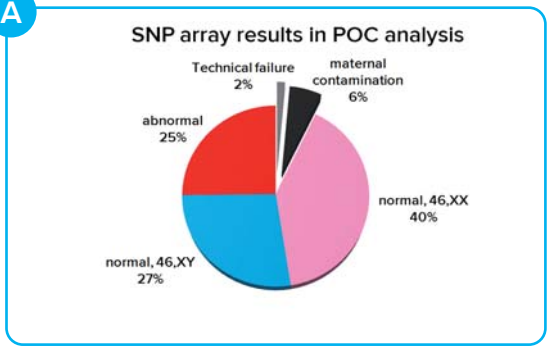
Fetal Death (FD) occurs in approximately 15% of clinically identified pregnancies. Cytogenetic abnormalities are present in 50% of spontaneous miscarriages (FD < 22 weeks of amenorrhea (WA) and in 6-13% of stillbirths (FD > 22 WA). Samples originating from these occurrences can be analysed by karyotype, CGH array or SNP array.

The advantage of SNP array over karyotyping cannot be refuted: the failure rate does not surpass 1% compared to the 40% failure rate of the karyotype (Figure 14A). In additional, the resolution is 50 times more defined in SNP array, which in turn increases the sensitivity and therefore the number of pathogenic abnormalities detected (Figure 14A).

The SNP array also has an advantage over CGH array : it enables the detection of triploidies, which represent close to 5% of FD < 22 WA, which is impossible in CGH array.

Figure 14B shows the case of a POC sample analysed by SNP array: fetal triploïdy resulted in a first trimester miscarriage.

A



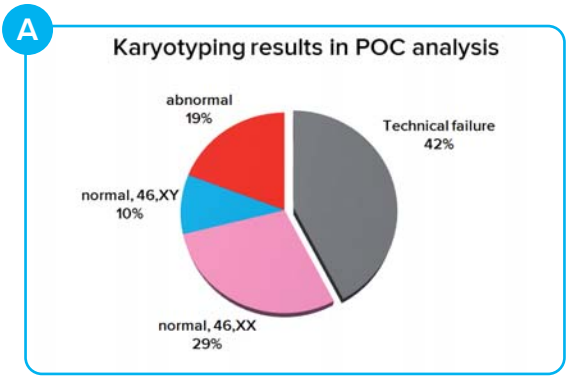


Figure 14A : Comparison of karyotype and SNP array performance in products of conception analysis.

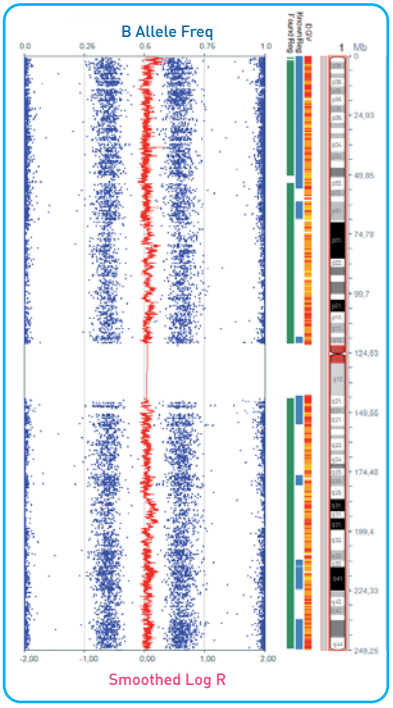


Figure 14B: SNP array result shows a triploïdy, here is the example of chromosome 1, all chromosomes have a comparable profile. Log R ratio has not deviated due to normalisation, triploïdy is visible thanks to genotyping data (B Allele Freq).

Highlights

Karyotyping provides an overall analysis of the human genome but the resolution of the test remains low compared to new techniques based on oligonucleotide arrays (CGH Array and SNP Array). These new technologies enhance the genetic etiological diagnosis of MR and CA (Figures 15 and 16). SNP Arrays provide a genome-wide study of all imbalanced genomic anomalies with a resolution close to 100 kb. The advantages of SNP Arrays are numerous:

- Overall analysis of the genome;
- Highlighting genomic changes that are not detected by karyotyping;
- Identification and characterisation of the loss of genomic material in the case of structural anomalies with an apparently balanced karyotype;
- Detection of the loss of heterozygosity, generally seen in cases of uniparental disomy;
- Identification and characterisation of the super-numerary chromosome markers seen in the karyotype;
- SNP Array does not require prior cell culture. The advantage of working directly with amniotic fluid avoids culture selections, and a decrease in the turnaround time from 10-15 days to only 1 week without parental confirmation.

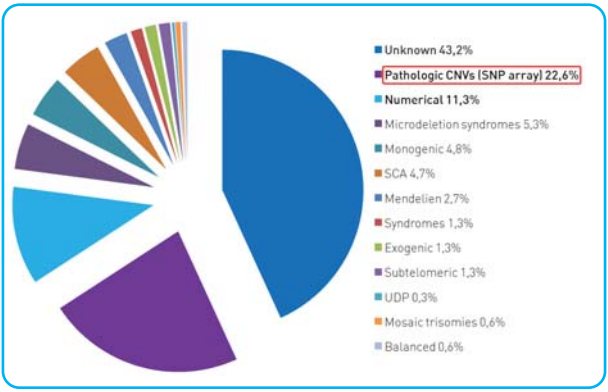
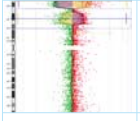
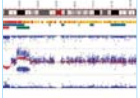
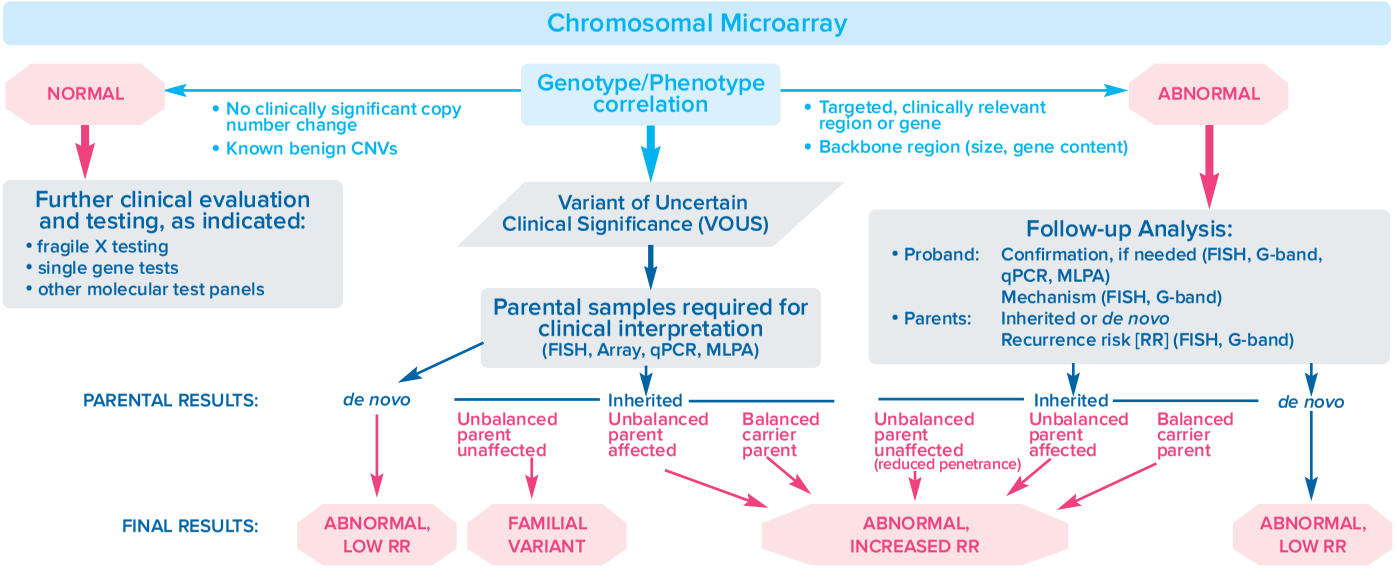


Figure 15. *Overview of distribution of various etiological causes of developmental delay and mental retardation[1-2].*

Figure 16. Advantages of SNP Array.

Output of method	Method	Resolution	Sensitivity	Genome Coverage	UDP detection	Non-Dividing Cells	Screening for new lesions	Unbalanced lesions	Triploidies
	Karyotype	5-10 Mb	10%	Complete	No	No	Yes	Yes >5-10 Mb	Yes
	FISH	150-200 Kb	Locus specific	Probe specific	No	Yes	No	Yes >150-200 Kb	Yes
	CGH Array	30-100 Kb	2-30%	Complete	No	Yes	Yes	Yes >30-100 Kb	Yes
	SNP Array	30-100 Kb	2-30%	Complete	Yes	Yes	Yes	Yes >30-100 kb	No

Clinical Genetic Testing: Patients with unexplained DD, MR, MCA, ASD*[18,19,20]



American College of Medical Genetics had recommended replacing karyotyping with Chromosomal Microarrays as “First-Line” Postnatal Test. Earlier this year, ISCA International Standards for Cyto-genomic Arrays Consortium was recommended using arrays as “first-tier” tests to assess individuals with unexplained Development Delay (DD) and Intellectual Disability, Autism Spectrum Disorders (ASD) or Multiple Congenital Anomalies (MCA). ISCA Consensus Statement. AJHG, 2010[18], International Guidelines[19,20]

Indications for SNP Array applications to clinical genetic testing

HumanCytoSNP-12 BeadChip is indicated for:

- Unexplained developmental delay or intellectual disability.
- Dysmorphic features or congenital anomalies
- Autism spectrum disorders, seizures or a clinical presentation suggestive of a Chromosomal syndrome.
- Patients with a normal chromosome analysis result and abnormal phenotype.
- Patients with suspected UPD.
- For unbalanced rearrangements, SNP Array can be used to size the deletion or duplication, or identify the genes involved and their content.
- For apparently balanced rearrangements and an abnormal phenotype, SNP Array can be used to test for cryptic deletions/duplications at the break-points.
- Infertility and sexual disorder development.

References

1. Rauch A, Hoyer J, Guth S, *et al.* (2006): Diagnostic yield of various genetic approaches in patients with unexplained developmental delay or mental retardation. Am J Hum Genet, (140A): 2063–2074.

2. Stankiewicz P, Lupski JR (2002): Genome architechture, rearrangements and genomic disorders. Trends Genet, 18:74-82.

3. Gijsbers AC, Lew JY, Bosch CA, *et al.* (2009): Anew diagnostic work flow for patients with mental retardation and/or multiple congenital abnormalities. Eur, J Hum Genet, 17(11):1394-402.

4. Pinkel D, Segraves R, Sudar D, *et al.* (1998): High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet. (20):207-211.

5. De Vries, BA Pfundt R, Leisink M, *et al.* (2005): Diagnostic Genome Profiling in Mental Retardation. Am J Hum Genet; 77(4): 606–616.

6. Devi A, Benn PA. X-chromosome abnormalities in women with premature ovarian failure. J Reprod Med 1999 ; 44 : 321-4.

7. Sherman SL (2000). Premature ovarian failure in the fragile X syndrome. Am J Med Genet; 97 : 189-94.

8. Murray A, Webb J, Crimley S, *et al.* (1998): Studies of FRAXA and FRAXE in women with premature ovarian failure. J Med Genet; 35 : 637-40.

9. Shaffer LG, Coppinger J, Alliman S, *et al.* (2008): Comparison of microarray-based detection rates for cytogenetic abnormalities in prenatal and neonatal specimens. Prenat Diagn; 28: 789–795.

10. Rickman L, Fiegler H, Carter NP, *et al.* (2005): Prenatal diagnosis by Array-CGH. Eur J Med Genet; 48: 232–240.

11. Rickman L, Fiegler H, Shaw-Smith C *et al.* (2006): Prenatal detection of unbalanced chromosomal rearrangements by Array CGH. J Med Genet; 43: 353–361.

12. Sahoo T, Cheung SW, Ward P, *et al.* (2006): Prenatal diagnosis of chromosomal abnorma-lities using array-

based comparative genomic hybridization. J Med Genet; 43: 719–727.

13. Friedman JM (2009): High resolution array genomic hybridization in prenatal diagnosis. Prenat Diagn; 29: 20–28.

14. Kleeman L, Bianchi D, Shaffer LG, *et al.* (2009): Use of array comparative genomic hybridization for prenatal diagnosis of f□tuses with sonographic anomalies and normal metaphase Karyotype. Prenat Diagn; 29: 1213–1217.

15. Van den Veyver IB, Patel A, Shaw CA, *et al.* (2009): Clinical use of array comparative genomic hybridization (aCGH) for prenatal diagnosis in 300 cases. Prenat Diagn; 29: 29–39.

16. Srebniak M, Boter M, Oudesluijs G, *et al.* (2011): Application of SNP Array for rapid prenatal diagnosis: implementation, genetic counselling and diagnostic flow. Eur J Hum Genet.; 19(12):1230-7.

17. Fiorentino F, Caiazzo F, Napolitano S, *et al.* (2011): Introducing array comparative genomic hybridization into routine prenatal diagnosis practice: a prospective study on over 1000 consecutive clinical cases. Prenat Diagn; 31(13):1270-82.

18. ISCA Consensus Statement: Building momentum: Arrays as first-line? AJHG, May 2010

19 : de Leeuw N, Dijkhuizen T, Hehir-Kwa J Y, et al. (2012): Diagnostic interpretation of array data using public databases and internet sources. Hum Mut; 33, 930–940.

20. ACMG standards and Guidelines, Genet Med, 2011 & 2013

21. Levy B, Sigurjonsson S, Pettersen B, et al. (2014): Genomic Imbalance in Products of Conception: Single-Nucleotide Polymorphism Chromosomal Microarray Analysis. Obstet Gynecol; 124, 202–209.

Sample requirements

Type	Specimen Requirements	Specimen Collection and Shipping
Whole Blood	5 ml EDTA whole blood *	Store and transport sample at room temperature
	*For paediatric samples, please also send EDTA samples of the parents	
Amniotic fluid	5 ml of amniotic fluid	Store and transport sample at room temperature
POC	Please also include maternal EDTA sample to exclude contamination	Store and transport sample at room temperature
TAT: 10 days		

Signed genetic consent form and detailed clinical informations are essential

Notes

Notes

Contacts

Author

Saïd EL MOUATASSIM, PhD

Molecular Genetic department, Biomnis Lyon

Saïd El Mouatassim, PhD
Grégory Egea, MD

Phone number: +33 (0)4 72 80 23 65
Fax number: +33 (0)4 72 80 23 66

Biomnis International Division

International Division
17/19 avenue Tony Garnier
BP 7322
69357 Lyon cedex 07
France
Phone number: +33 (0)4 72 80 57 42
Fax number: +33 (0)4 72 80 73 56
E-mail: international@biomnis.com
Website: www.biomnis.com/international

DS21-INTGB - FEBRUARY 2016 - Crédit photo : 123RF/zentilia