ARRAY CGH IN SECOND OR THIRD TRIMESTER FETUSES WITH MAJOR STRUCTURAL ABNORMALITIES AND/OR INTRAUTERINE GROWTH RESTRICTION

Maddalena Santirocco

ARRAY CGH IN SECOND OR THIRD TRIMESTER FETUSES WITH MAJOR STRUCTURAL ABNORMALITIES AND/OR INTRAUTERINE GROWTH RESTRICTION

Doctoranda:

Maddalena Santirocco

Directores de Tesis:

Dra. Nerea Maiz Elizaran,

Doctora en Medicina por la Universitat de Barcelona

Dr. Alberto Plaja Rustein

Doctor en Biologia por la Universitat Autònoma de Barcelona.

Tutor de Tesis:

Dr. Lluís Cabero Roura

Catedrático emérito del Departamento de Obstetricia y Ginecologia de la Facultad de Medicina de la Universitat Autònoma de Barcelona

Departament de Pediatria, Obstetrícia Ginecologia i Medicina Preventiva

Facultat de Medicina- Universitat Autònoma de Barcelona . Barcelona, 2022

Doña Nerea Maiz Elizaran

CERTIFICA

Que la tesis Doctoral "ARRAY CGH IN SECOND OR THIRD TRIMESTER FETUSES WITH MAJOR STRUCTURAL ABNORMALITIES AND/OR INTRAUTERINE GROWTH RESTRICTION", elaborada por Maddalena Santirocco y dirigida por la abajo firmante es apta para ser defendida ante el tribunal correspondiente, para optar al título de Doctor en Pediatria, Obstetricia y Ginecología, otorgado por el Departamento de Pediatría, Obstetricia y Ginecología y de Medicina Preventiva y Salud Publica de la Facultad de Medicina de la Universitat Autònoma de Barcelona, y para que conste a los efectos oportunos, firma la presente.

Nerea Maiz Elizaran

Don Alberto Plaja Rustein

CERTIFICA

Que la tesis Doctoral "ARRAY CGH IN SECOND OR THIRD TRIMESTER FETUSES WITH MAJOR STRUCTURAL ABNORMALITIES AND/OR INTRAUTERINE GROWTH RESTRICTION", elaborada por Maddalena Santirocco y dirigida por el abajo firmante es apta para ser defendida ante el tribunal correspondiente, para optar al título de Doctor en Pediatria, Obstetricia y Ginecología, otorgado por el Departamento de Pediatría, Obstetricia y Ginecología y de Medicina Preventiva y Salud Publica de la Facultad de Medicina de la Universitat Autònoma de Barcelona, y para que conste a los efectos oportunos, firma la presente.

Alberto Plaja Rustein

Doctoranda, Maddalena Santirocco
En Barcelona, febrero de 2022

A Lelippo e Babá

Per insegnarmi ogni giorno cosa sono il coraggio e la pazienza.

Agradecimientos

A la Doctora Carreras, por su acogida durante mi estancia, su humanidad y enorme profesionalidad, por haberme direccionado hacia mi carrera profesional como ginecóloga especialista.

A Nerea, por su ayuda inmensa en estos años, no solo durante mi proyecto de tesis. Por enseñarme el rigor cientifico y la pasión por la investigación. Por las pacientes horas pasadas juntas conmigo y mis bebés como becarios en su despacho revisando mi trabajo.

A Alberto, gracias por tu cercanía y disponibilidad constante, no obstante el enorme cargo de trabajo que le he significado. Si al final conseguí aprender algo de Genetica es gracias a su dedicación.

A Teresa Higueras, por haberme hecho sentir siempre parte del grupo, sobretodo cuando llegué.. una italiana recién aterrizada con muy poco conocimiento del idioma. Por formarme y demostrame lo importante que es no perder nunca la curiosidad y la pasión por nuestra especialidad. (y por enseñarme a hacer ecografías con ambas manos en cualquier situación).

A Silvia Arévalo y Carlota Rodó, por todo lo que me han enseñado trabajando codo a codo (accentos incluidos..), por toda la formación profesional. Pero también por la amistad y confianza que siempre han depositado en mi. Lograron hacerme sentir parte de una familia profesional, aquella que perdí al dejar mi Pais.

A Tamara, por su verdadera amistad.

A Manuel y Ana, por todas la risas, las discusiones, las comidas, y las noches que pasamos juntos, los disminutivos, los consejos, y el estress compartido.. Habeis sido lo más.

A Laura Y Mavalle, mis chicas.. Estos últimos meses hubieran sido muchos más pesados sin sus abrazos de los lunes, sin nuestras charlas de los jueves, y los planes de los viernes.

A Juli, por haber tomando el mando del barco de piratas de mi familia durante estos últimos meses.

A Silvia Vigo, sai che sarò sempre il tuo +1. anche dall'altra parte del mondo.

A Rebecca, la ginecologia e un bellissimo giro del destino ci hanno fatte trovare, grazie per l'entusiasmo e per credere in tutte le cose che faremo.

A voi, Mamma e Giuli, che mi supportate (e sopportate) da sempre, per tutte le volte che mi avete sostenuta e aiutata in questi anni, avanti e indietro tra Stati, con aerei, macchine, bambini (e a volte gatti), valigie con sapore di casa e amore incondizionato.

A tutta la mia famiglia, per esserci sempre e da sempre, senza mai cambiare.

A Gabri, ti prometto (o almeno ci provo) che è l'ultima tesi che mi vedi scrivere. Probabilmente sei l'ultimo che ringrazio perché sei la persona che mi consente di non dover scegliere, con cui non devo affrontare nessun test. Nessuna risposta multipla, nessuna crocetta, solo un tema libero, senza giustificazioni. Una traccia che si scrive libera, senza mai finire l'inchiostro.

Ai miei figli, un giorno, quando diventerete quello che vi farà sentire felici, capirete perché.

“ Il corpo faccia quello che vuole, io sono la mente”.

Rita Levi Montalcini

SUMMARY

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

AC	Amniocentesis
ADPKD	Autosomal Dominant Polycystic Kidney Disease
ARPKD	Autosomal Recessive Polycystic Kidney Disease
ASD	Atrial Septal Defect
BMI	Body Mass Index
CDH	Congenital Diaphragmatic Hernia
CEIm	Comité de ética de investigación con medicamentos
CGH	Comparative Genomic Hybridization
CHD	Congenital Heart Disease
CMA	Chromosomal Microarray
CNS	Central Nervous System
CNV	Copy Number Variants
CPAM	Congenital Pulmonary Airway Malformation
CVS	Chorionic Villus Sampling
DWM	Dandy Walker Malformation
HLHS	Hypoplastic Left Heart Syndrome
HRHS	Hypoplastic Right Heart Syndrome
IQR	InterQuartile Range
ISCN	International System for Human Cytogenetic Nomenclature
IVF	In Vitro fertilization
Kb	Kilobases
Mb	Million pair bases

MRI	Magnetic Resonance Imaging
NIHF	Non-Immune Hydrops Fetalis
NIPT	Non-invasive Prenatal Test
NTD	Neural Tube Defect
Pathogenic CNV	Pathogenic Copy Number Variant
PDA	Patent Ductus Arteriosus
T13	Trisomy 13
T18	Trisomy 18
T21	trisomy 21
TGA	Transposition of great Arteries
TOF	Tetralogy Of Fallot
TS	Turner syndrome
UPD	Uniparental disomy
UTD	Urinary Tract Dilation
VACTERL	(Vertebral anomalies, Anal atresia, Cardiovascular anomalies, Tracheo Esophageal fistula, Renal anomalies, and Limb defects) association
VSD	Ventricular Septal Defects
VUS	Variant of Unknown Significance

PART 1

INTRODUCTION

1 INTRODUCTION

Prenatal diagnosis, diagnosis of pregnancy loss and post-mortem evaluation often involve genetic evaluation. Cytogenetic analysis can be performed on amniocytes, fetal blood cells or chorionic villi cells.

Microscope observation of human chromosomes or karyotype analysis (mainly with G-banding techniques) has been considered for a long time the gold standard of genetic analysis. Standard karyotype allows the detection of aneuploidies and large structural changes in all chromosomes including balanced reorganizations (mainly inversions, translocations, and insertions) and unbalanced reorganizations (mainly gains or losses of chromosome material and more complex reorganizations as translocation unbalanced derivates, inversion recombinants, ring chromosomes and extra structurally abnormal chromosomes also named ESACs).

However, this technique has serious limitations: resolution, since it is unable to detect submicroscopic genomic alterations (<3-10 million pair bases [Mb]), need of a cell culture (requiring viable cells and a minimum of 10 days to obtain an adequate number of separating cells) [1]and a subjectivity and strong dependence on the ability of the analyst who performs the study under the microscope.

Chromosomal Microarray (CMA) studies the whole genome, searching for DNA copy number variants (CNVs) from 50-100 kilobases [Kb] well below karyotype resolution to chromosomal level,[2] and it is the actual gold standard for CNV detection in a clinical setting. [3,4] Its main drawbacks are the inability to detect balanced anomalies (like other molecular techniques including NGS) and that its resolution does not reach the sequence

level (which prevents detection of small mutations including sequence variants and the evaluation of phenomena such as changes in the reading frame).

DNA sequencing, with traditional Sanger or the recently developed NGS technologies allow to detect sequence variants and small copy number changes. NGS promises detection of larger CNVs as well in a near future. However, until now NGS have not reach enough reliability to replace array technology in the detection of larger CNVs.

1.1 INVASIVE PRENATAL TESTS

Throughout gestations, cells suitable for genetic testing are collected from the amniotic fluid by amniocentesis (AC), trophoblastic cells by chorionic villus sampling (CVS), or fetal blood or tissue derived from the direct biopsy. Fetal cells can also be obtained from maternal blood and pre-implantation embryos (in this last situation, however, in vitro fertilization is required); however, the only accurate diagnosis is made through a direct fetal or placental cells sample.

Recent evidence suggests that the major risk of miscarriage reported being associated with invasive procedures has to be found in the indication for the invasive test itself and not in the technical procedure: the etiologic genetic alteration or the condition that led to an invasive test would be the real cause for the miscarriage or the fetal loss, more than the procedure performed to achieve the diagnosis; considering that, the real risk of miscarriage associated with the procedure (either amniocentesis or CVS) is reported to be low in gestations without risk factors, being comparable or only slightly higher than the risk reported for the general population. [5]

1.1.1 CHORIONIC VILLUS SAMPLING

Chorionic villus sampling (CVS) is a prenatal test performed in the first trimester, where a biopsy of chorionic villi from the placenta is done with the aim of genetic testing. It consists of the withdrawal of trophoblastic cells from the placenta; the sample can be taken through the cervix (transcervical CVS) or the abdominal wall (transabdominal CVS); it usually is performed between 11 and 13+6 weeks of gestation. A chorionic villus sample is obtained by inserting a transabdominal needle or a transcervical cannula aspiration or biopsy forceps, both procedures with direct ultrasound guidance .The type of procedure depends on the operator's experience and preference, the gestational age, and the placenta location. [6]

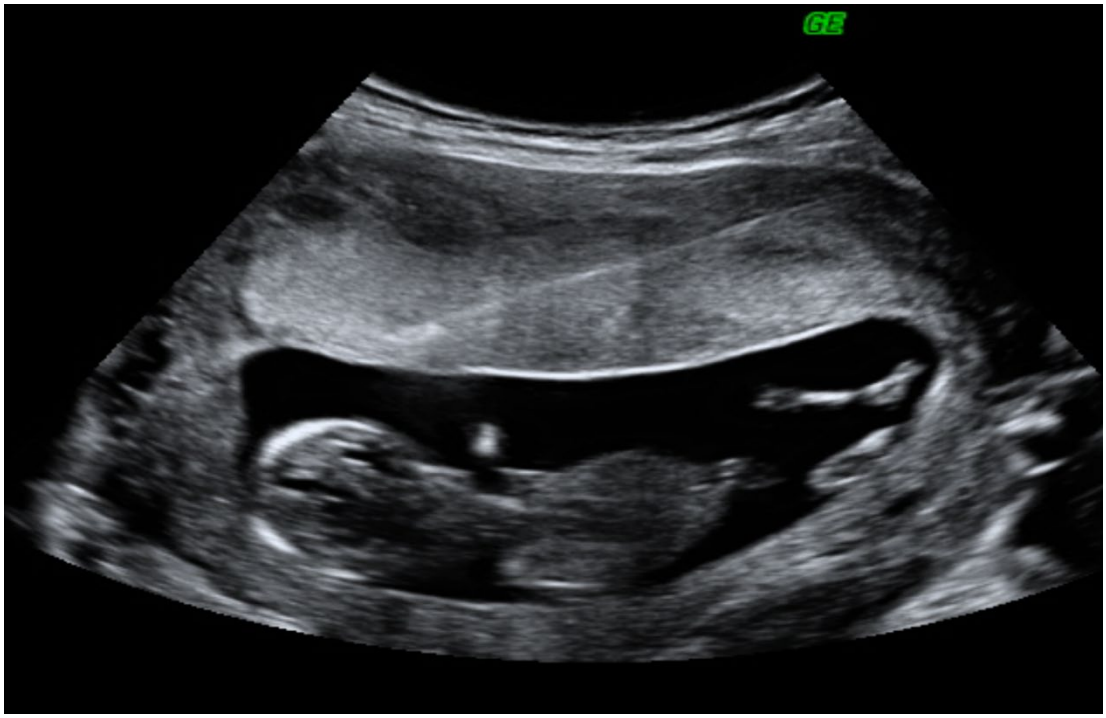


Figure 1 Transabdominal chorion villus sampling

A Randomized controlled trial performed in 3873 singleton pregnancies showed no significant difference between the two methods in terms of fetal loss and successful sampling [7].

The risk of fetal loss after a CVS is reported to be around 0.2-2 %. The operator's experience is one of the most important modifying factors; repeated needle insertions and a low gestational age are also associated with more complications. According to a recent meta-analysis, the rate of fetal loss after CVS is not increased significantly compared to the non-exposed population, with a rate of a miscarriage of 0.22%.[8] A Danish report of 2016 found practically no impact of the CVS technique on fetal loss rate, with a risk of a miscarriage of 0.21 % 21 days after the procedure. [9]

The procedure is not indicated at a very early gestational age (before 10 weeks) due to a higher risk of complications and fetal loss. Several studies reported an increased incidence of specific fetal anomalies, such as oromandibular hypoplasia and limb reduction, in fetuses where a CVS had been performed before 10 weeks of gestation, compared to general population. [6,10]

The diagnostic accuracy of chorionic villus sampling is reported to be around 97.5 % and 99.6%, mostly due to the possible presence of placental mosaicism. A failure in the trophoblastic culture is reported to occur in less than 0.5% of procedures, being maternal decidual cells contamination one of the possible causes. Up to 1% of the procedures investigated with classical cytogenetic techniques can present placental cell mosaicism: in this case, a genetic consultation is required to rule out true fetal or placental confined mosaicism. [11]

1.1.2 AMNIOCENTESIS

Amniocentesis is a technique for obtaining amniotic fluid from the uterine cavity using a needle, under continuous ultrasound guidance, via a transabdominal approach (Figure 2). A sample of fetal exfoliated cells, transudates, urine, or secretions can be obtained.

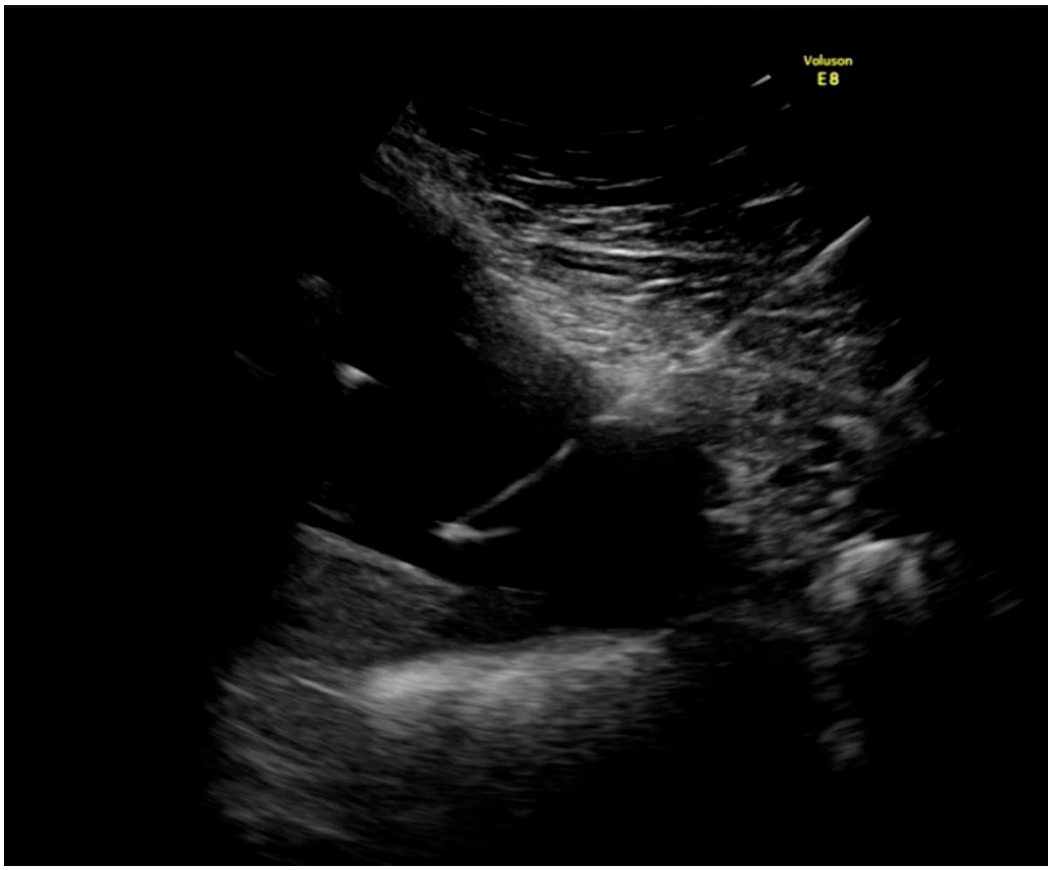


Figure 2 Amniocentesis needle inside the amniotic cavity.

Different studies can be performed on the amniotic fluid sample, including chromosomal, molecular, biochemical, and microbial studies.

Traditionally it is performed starting from 16 weeks of gestation to minimize the risk of fetal damage and obtain enough viable fetal cells; a possible complication when amniocentesis is performed at a very early gestational age (<15 weeks) would be the

possibility of provoking neonatal orthopedic (such as talipes) and respiratory anomalies (respiratory distress syndrome). Moreover, it is reported a success rate of 82% if performed before 15 weeks compared to 94% if obtained at 16 weeks or later. Data back from the 1970s demonstrated that at this gestational age, a large amount of amniotic fluid could be aspirated with reduced technical difficulties.

A disadvantage of a late amniocentesis could be that the final result will be obtained after 17 weeks of gestation, with a long waiting period that can be distressing for families. Moreover, results could be available after the legal limit for termination of pregnancy (TOP), which varies from country to country. Earlier options are chorionic villus samplings.

The most frequent reasons for amniocentesis are to allow diagnosis of chromosomal anomalies, single-gene disorders, fetal infections, intra-amniotic inflammation status in a prenatal setting, and assess fetal lung maturity, blood, and platelet different types.

The procedure carries a risk of fetal loss due to the invasive nature of the test; when performed in the second trimester of gestation, the amniotic membrane has fused with the chorion, the risk of fetal loss is reported to be around 0.5 % (range, 0.06–1%).[9,12]

Furthermore, a risk of amniotic fluid leakage is described in approximately 1-2 % of procedures. Literature reports other rare complications such as placental hemorrhage, intra-amniotic infection, abdominal wall hematoma and possible direct fetal lesion.[13]

In a meta-analysis conducted by Akolekar et al., the risk of miscarriage after amniocentesis was reported to be around 0.11 %, being not significantly different between the control and invasive test groups. The authors postulate that the rate of

fetal loss would be overestimated due to some gestation characteristics that increase the risk of chromosomal alterations (and secondly, the number of invasive tests) and could be related to an increased risk for miscarriage. In the same meta-analysis, significant heterogeneity between studies is reported, reducing the robustness of the analysis itself. Moreover, as the prenatal diagnosis has moved into the era of cell-free DNA tests, this would lead finally to a decrease of invasive tests performed, making it difficult to have very good-quality studies published on the real risk of fetal loss following invasive procedures. [14]

There is no good quality evidence to support some extra recommendations for the procedure, so most authors suggest performing amniocentesis using those methods and techniques the operators are most familiar with.[15,16.]

1.1.3 ELIGIBILITY FOR CHORIONIC VILLUS SAMPLING OR AMNIOCENTESIS

Before any invasive procedure, the operator should provide to the patients detailed counselling regarding benefits, possible risks, limitations, and technical aspects of the procedure. A pre-test counselling is required, and it should be conducted by a specialist in obstetrics or maternal-fetal medicine who will perform the procedure or by a geneticist.

The professional should explain the advantages and risks of an invasive prenatal test (either CVS or amniocentesis) vs a screening, differences between the invasive test procedures, including the rate of pregnancy loss, estimated time and accuracy of results of laboratory tests that will be eventually performed, the possibility of an uncertain or inconclusive result and or secondary finding (a pathogenic result not related to the

reason of the analysis) and the method of communication of the results. At the end of the pre-test counselling, written informed consent should be obtained from the patient.

Common indications for amniocentesis or a chorionic villus sampling are:

- Increased risk of fetal aneuploidy: the risk may derive from first or second-trimester screening test (including 1st trimester combined test, non-invasive prenatal test (NIPT), 2nd-trimester biochemistry test), ultrasound detected anomaly, obstetrics or family positive history for fetal chromosomal anomalies (e.g., male status of a fetus of a pregnant woman for an X-linked disease, carrier status of both parents for an autosomal recessive (AR) disorder).

- Increased risk for a known genetic or biochemical disease; the risk may be due to a disease with a familiar inheritance caused by a known mutation or a biochemical change.

- Infectious diseases: if a primary infection or a seroconversion during gestations occurs involving toxoplasma, cytomegalovirus or rubella, an invasive test is advocated to rule out or confirm fetal transmission of the infection.

- Maternal request: usually due to intense parental anxiety or advanced maternal age, depending on specific countries' policies.

Post-test genetic counselling is mandatory in case of abnormal or uncertain results.

1.2 CHROMOSOMAL MICROARRAY

For about 50 years, cytogenetic analysis of G-banded human chromosomes has been the gold standard in postnatal and prenatal genetic analysis of individuals with

malformations. However, cytogenetic analysis has a low resolution (approximately 5-10 Mb in size[17]), requires a cell culture, a slow and delicate laboratory procedure, and interpretation; in some cases is subjected to some subjectivity and has a strong dependence on the ability of the analyst who performs the study under the microscope. Chromosomal Microarray (CMA) is a molecular cytogenetic technique used for the diagnosis of genetic imbalances on every chromosome, in the kilobase (kb) range resolution or, even, in some cases, at the exonic level[2], without a cell culture. Because its superior resolution and diagnostic yield, CMA has replaced traditional cytogenetic techniques as a first-tier approach for the postnatal evaluation of individuals with intellectual disability, developmental delay, autism spectrum disorder, and/or multiple congenital anomalies, as well as for prenatal evaluation of fetuses with structural anomalies observed by ultrasound [18]

1.2.1 COPY NUMBER VARIANTS

The definition and existing knowledge of the various types of genetic variability strongly depend on the technology involved in its detection. In this line, chromosomal abnormalities are defined as changes in the structure or number of chromosomes, balanced or not, visible under an optical microscope (usually more than 5-10 Mb and never less than 3 Mb). However, new molecular and cytogenetics techniques have revealed that variations in the structure of less than 3 Mb are extremely frequent, and it has defined the term “structural variation” as changes in the structure of the chromosomes, balanced or not, greater than or equal to 50 bp (the detection limit of the techniques of NGS). Genomic structural variation

includes microscopic and submicroscopic types, such as deletions, duplications, copy-number variants, insertions, inversions and translocations.

Copy Number Variations or CNVs are a special type of structural variation detected by array technology: unbalanced gains or losses equal or greater than 1000 Kb.

As technology has been improving, arrays currently detect CNVs of less than 1000 Kb and NGS anomalies of more than 50 bp, so it is likely that in the future, it will be redefined and unify terminology and detection technology.

Although estimates depend on the technical approach involved, the average number of CNVs detected per genome is 70, and the mean size is 341 kb. [19,20] Most CNVs are not clinically significant and are found in apparently normal individuals. This kind of CNVs are defined as "benign" and do not contain or interfere with the proper function of significant coding regions sensible to doses (genes that need exactly two copies to function properly). Although they are frequently very small in size, many examples of benign CNVs in the range of Mb are known. The current evidence is that all individuals carry CNVs, the number depending on the resolution of the technique employed, on average three large-scale CNVs [21] to hundreds.

The relevance of CNVs in medicine depends on the possible phenotypic effect that the microdeletion/duplication is likely to produce when the genetic imbalance occurs in a part of coding DNA containing critical genes or regulatory regions.[22] The number of diseases causing CNVs is continuously increasing.

CNV distribution is non-random across the genome; both hot and cold spots have been reported. The frequency is higher in those regions of segmental duplication (4-10 times

more frequent) with nonallelic recombination as a mechanism for the production of CNVs.[23] CNV are more common in gene-rich DNA regions; they are observed more frequently in specific gene families such as immune and inflammation response genes, cell adhesion molecules, olfactory receptors, and structural proteins.

Pathogenic CNVs include dose-sensitive genes. The term haploinsufficiency is used with genes in that a single copy of the wild-type allele at a locus in heterozygous combination with a variant or loss allele is insufficient to produce the wild-type phenotype. Some pathogenic CNVs cause disorders with reproducible phenotypic features (e.g., Williams syndrome caused by deletions of the elastin gene, Charcot-Marie-Tooth disease type 1A [CMT1A] due to duplications of the PMP22 gene); others are associated with susceptibility to disease (e.g., cancer, HIV, infection, autoimmune disorders, autism spectrum disorders).[24] In some cases, Mendelian diseases can be produced when large losses of genetic material include the appropriate haploinsufficient gene or even when small losses or gains at the exonic level inside the gene disruption. CNVs are also described in complex syndromes or traits in which a combination of genetic and environmental factors are present .[25] For some conditions, a contribution of multiple CNVs could explain the different phenotypic expression: a retrospective postnatal study in which array comparative genomic hybridization (CGH) was performed in 2312 children with developmental delay known for having one CNVs, found that, compared with a control group of 8329 children without developmental disabilities, the first group presented a higher number of second site CNVs. The CNVs might have been the cause of developmental alterations or be considered markers of susceptibility to genomic damage.[26]

Causes of CNVs are the erroneous pairing of highly homologous DNA regions that cause misalignment and unequal recombination during meiosis; this mismatch can lead to duplication and deletion of part of chromosomes resulting in CNVs, a process called nonallelic homologous recombination (NAHR).[27] Other mechanisms include nonhomologous end joining and microhomology-mediated break-induced replication. It is not known what factors predispose some individuals to develop these changes, although CNVs are more like to arise de novo in syndromic disorders. Conversely, CNVs are more likely to be inherited in conditions with variable phenotype (e.g., intellectual disability). A possible explanation may be that reproductive fitness is reduced in individuals with severe syndromic disorders.[26]

Interpretation of CNVs relies upon extensive bibliography research and the use of international databases that permit a comparison with apparently normal controls, like the Database of Genomic Variants (DGV), the 1000 Genomes Project [28], and phenotypically abnormal patients (like ClinVar [29] and DECIPHER (Database of Chromosomal Imbalance and Phenotype in Humans using Ensembl Resources))[30]. DECIPHER is an interactive web-based database that incorporates a set of tools designed to interpret genomic data variants (CNVs but also sequence variants). It involves more than 270 centers around the world, with more than 36,000 cases uploaded. For each contributing center there is a rare diseases clinician or clinical geneticist who is responsible for controlling data entry. Another platform is **The Clinical Genome Resource (ClinGen)**/ International Standards for Cytogenomics Array (ISCA) consortium, which provide regular reviews and updates on haploinsufficiency or triplo-sensitivity scores assigned to genes.[18]

Since CNV detection is technology dependent and endemism's exist, CNV data from the laboratory performing the study is also of paramount importance in correct CNV interpretation.

Finally, cytogenetic and CNV results are usually described with an "ISCN formula" in a short and a standard nomenclature named "International System for Human Cytogenetic Nomenclature (ISCN)", an international standard for human chromosome nomenclature, which includes band names, symbols and abbreviated terms used in the description of human chromosome and chromosome abnormalities. The International System for Human Cytogenetic Nomenclature (ISCN) includes ideograms with band names, symbols, and abbreviated terms utilized to describe human chromosome and chromosome abnormalities. It provides a standard nomenclature for describing genomic rearrangements identified by karyotype, FISH, MLPA, microarray, and DNA sequencing. A description of a CNV is provided by a formula that indicates the genome's version, the chromosome, chromosomic band, genomic coordinates (first and last nucleotide involved in the chromosomal abnormality), and the number of copies. The current version is ISCN (2020); S. Karger Publishing. ISBN 978-3318068672. [31]

1.2.2 COUNSELLING AND ETHICAL ASPECTS

Implementing the CMA in the postnatal field demonstrated to increase the diagnostic yield compared with standard karyotype, giving a clear benefit to families and clinicians in terms of management, diagnostic information, and proper counselling of recurrence risk. In a prenatal setting, the benefits derived from the increased diagnostic cannot be underestimated. In this case, it allows families to have specific information about the nature of the condition affecting the fetus and its possible prognosis and to make an

informed decision regarding the pregnancy. Moreover, it is possible to perform precise recurrence risk counselling for future gestations.

When the technology was implemented, concerns were raised about the bio-ethical principle of no maleficence; array may cause harm for producing findings that can impact decision-making, provoking great anxiety and possibly violating the future child's juridical rights. CMA applied to the prenatal field can produce some incidental discovering, including CNVs causing late-childhood or adult-onset diseases, cancer predispositions (such as mutation of BRCA1/BRCA2 genes which confer a risk of breast and ovarian cancer), CNVs with not known or uncertain significance (VUS), and recurring CNVs of incomplete penetrance that are known to create a predisposition to neurologic and psychiatric conditions.[32] However, this problem is not exclusively array related since also with a standard karyotype, it is possible to encounter findings of uncertain clinical impact (e.g. extra markers chromosomes or "de novo" apparently balanced rearrangements[32] A meta-analysis conducted by Hillman found a prevalence of not clear findings in 1.1% of all cases and 1.9% in cases with one or more ultrasound anomalies.[33]

Another concern is that receiving a pathogenic or uncertain result could provoke anxiety in the parents and thus harm the development of a proper bond with the future child.[34] Uncertain findings also create great concern and responsibility for clinicians and those who oversee the counselling. A study conducted by Bernhardt *et al.* explored women's point of view when they received abnormal prenatal results derived from CMA and found that some women who had received uncertain array results continued to worry not only in the pregnancy but also after the delivery and had regrets about having had an array test.[35]

Moreover, an abnormal array result in a fetus usually is followed by testing in relatives to investigate the clinical significance; this is an additional burden that families must face. Those CMA anomalies that are found to be inherited can leave the parents with the difficult feeling that they carry a CNV, which is usually present in people with health problems. [35]

Another issue, given the wide range of possible results derived from a CMA, is the fact that it can be difficult to assure that parents are expressing truly informed consent.

To overcome these aspects, different strategies have been proposed to minimize the possible maleficence of array, maximizing its benefits for the prenatal setting.

-limitation of reporting: it has been suggested to withhold all the information that is not crucial for the decision making and to inform only the information directly related to the anomaly; one ethical dilemma is whether to inform or not those CNVs associated with increased risk for cancer (like BRCA1 and BRCA2 deletions). [36] If it is decided not to inform, a particularly important consideration would be if there will be an opportunity to disclose later when it becomes of relevance. Genetics changes are heritable, so attention must be given to whether the information could be relevant to the future child or adults health related to the fetus (BRCA changes may be of immediate importance for the mother).

- limitation of diagnosing: it refers to modified arrays with a special design adapted for the use in the prenatal setting; some arrays are modified to increase detection of already known microdeletions and duplications and loci of inherited disorders. With this approach, the risk of a VUS finding is minimized, but novel or rare significant imbalances

may be missed; moreover, in this way, parents' autonomy may be violated if they were not specifically explained that a modified array would be used, and uncertainty information could be considered a legitimate factor in the process of decision-making.[37]

-limitation of access is one of the most used strategies. It consists of restricting access to an array to specific groups of patients. Against this approach could be argued that some conditions detectable with CMA have a comparable degree of severity with trisomy 21 but may present without ultrasound anomalies, and in this scenario, they would be missed. A group reported that when given a choice between array and standard karyotype, 70% of patients chose array over conventional karyotype (chosen by only 30% of the patients).[38]

Belgian approach

Because of the big debate generated with prenatal CMA implementation, in 2014, all 8 genetic centers in Belgium elaborated a unique project with a national consensus approach on how microarray was implemented.[39] Belgian geneticists agreed on using an array with a fixed resolution of 400 kb for all indications of invasive testing for both deletions and duplications to minimize the number of VUS while maximizing the detection of pathogenic variants. Guidelines on how to report and inform CNVs were redacted, and a shared database for the prenatal array was created, the Belgian MicroArray Prenatal (BEMAPRE) database relating prenatal genetic and ultrasound findings to postnatal clinical data.[40]

In Belgium, CNVs are classified as benign, pathogenic, susceptibility or VUS; all the variants that are not classified in one of the first three are classified as VUS and are not

reported. The classification is under continuous review of the Belgian Society for Human Genetics (BeSHG) Prenatal Committee,[41] which is a collaboration of all the clinical and laboratory geneticists from Belgium. The Belgian approach created a worldwide discussion; it brings the advantage to prevent inconsistency between centers and avoids parental anxiety in the case of VUS; on the other side, it can be considered as paternalistic since it does not leave the patients the final decision about what to know, moreover it could have legal implications because non-communicated VUS could become relevant in the future.

The diagnosis of a VUS creates a substantial ethical and counselling challenge in a prenatal context, in which there is not a fetal phenotype guiding the interpretation of a doubtful array result. In the first period of CMA implementation, this led to controversy about whether or not to extend the use of microarray further than those pregnancies where a fetal anomaly was detected on ultrasound.[42] Guidelines were slow to appear, in 2016 the American College of Obstetrics and Gynecology (ACOG) and the Society of Maternal and Fetal Medicine (SMFM) stated that either standard karyotype or microarray could be performed when an invasive test is programmed, even if the fetus does not present a structural anomaly.[4] Belgian collective guidelines recommended the use of array for any indication and implemented a clear reporting policy that excludes VUS, but also provide an online specific experts committee to consider ambiguous cases.[39] The English guidelines, on the other side, support the use of array in case of fetal structural abnormality and/or nuchal translucency (NT) above 3.5 mm but remain silent about other indications. Regarding VUS, they recommend that those VUS apparently not related to phenotypic effect should not be reported.[43] The Australian

guidelines support the use of CMA in all those contexts in which a fetal anomaly is detected but do not mention other indication groups; at the same time, the importance of genetic counselling for pathogenic CNVs and VUS is highlighted.[44]

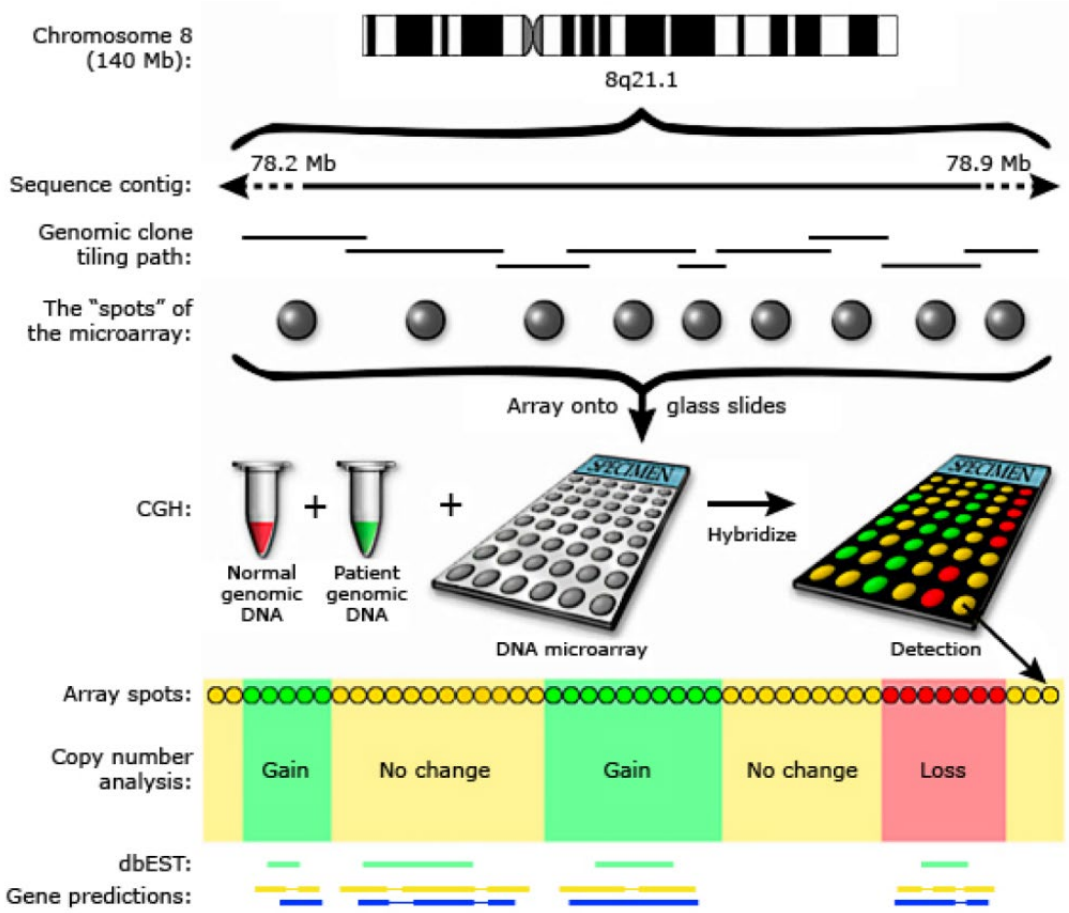
1.3 ARRAY COMPARATIVE GENOMIC HYBRIDIZATION (CGH)

Array comparative genomic hybridization (array CGH), also known as chromosome microarray or microarray-based comparative genomic hybridization, is the gold standard laboratory test for detecting CNVs that cause genomic disorders.

Array CGH allows detection of small losses or gains of genomic material down to several kilobases (kb) and even at the exonic level.

1.3.1 TECHNIQUE

There are currently two basic array technologies for detecting CNVs: CGH (Comparative Genome Hybridization) Arrays and SNP (Single Nucleotide Polymorphism) Arrays. The technology used in our hospital (CGH array) allows the detection of small losses or gains of genomic material comparing the genomic content (DNA) of a patient to that of a normal control, identifying areas with different copies. In brief, DNA from the patient and a phenotypically normal are fragmented and labeled with different fluorochromes (Cy5 red and CY3 green/blue fluorescence). Both DNA are mixed and hybridized to probes (short fragments of DNA) attached to a glass surface. The sequence of each probe is complementary to one site in the human genome, and therefore a single array experiment investigates thousands of DNA sites. If probes are distributed throughout the genome, array technology detects all gains and losses from very large, including aneuploidy of entire chromosomes, to very small (below the 400-200 Kb range).



Beheshti, B., Park, P. C., Braude, I., & Squire, J. A. (n.d.). Microarray CGH. Molecular Cytogenetics 2002, 191–208.

Figure 3: Array CGH setup

The intensity of both fluorescence colors is measured with a digital imaging software and utilized to calculate the ratio of the control and the patient's DNA.

Assuming control DNA has a normal copy number, a ratio of one expresses an equal contribution from the two samples and represents a normal copy number at the considered locus; a ratio greater than one reflects more of the patient's DNA represented compared to the control DNA; this represents a gain of material. A ratio less

than one indicates more hybridization of the control DNA compared to the patient's DNA, meaning a loss of genetic material in the patient's DNA (deletion or monosomy).

CMA is designed to quantify the amount of DNA present; therefore, it is unable to detect the exact location of the extra material (as in a duplications) and cannot detect balanced chromosome changes, which do not result in net gains or loss of genetic material. (e.g., translocations, inversions or balanced mosaics as X/XXX).

In a prenatal setting, CMA is performed on fetal DNA samples derived from uncultured or cultured amniocytes, fetal blood or chorionic villus cells.

The first array-CGH platforms used large-insert clones, such as BAC (Bacterial Artificial Chromosomes) derived from the Human Genome Project. BAC probes vary in length from 150 to 200 kb.

BAC arrays generate an intense hybridization signal with a high signal-to-noise ratio. This translates to a robust, reproducible assay, making them a good choice when the array was first implemented for the poor quality DNA often found in non-cultured prenatal samples.[45] Nevertheless, BAC platforms are not cost-effective for a laboratory accepting many prenatal samples and have a very low resolution compared to oligonucleotide based arrays (about 1Mb) because have few printed elements on the arrays and BAC probes have a large size. [46]

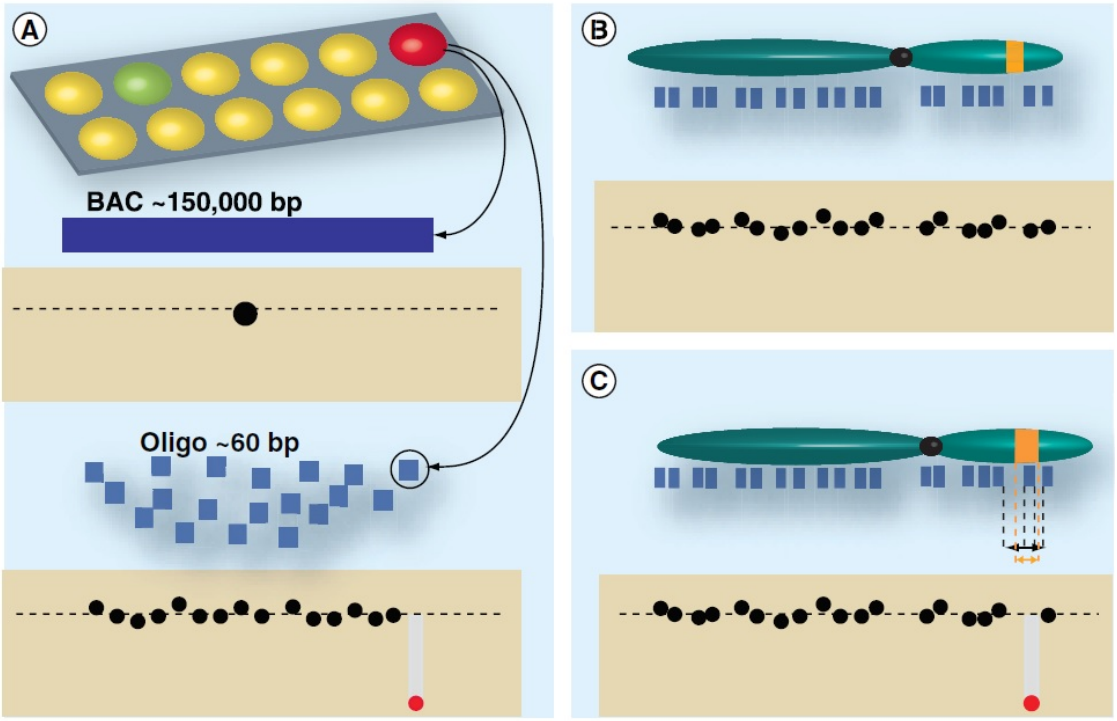
In recent years, BAC arrays have been totally replaced by oligo-based platforms, CGH arrays or Single Nucleotide Polymorphism (SNP), for the higher resolutions these platforms offer.

1.3.2 OLIGONUCLEOTIDE ARRAYS

Oligonucleotide (OligoCGH) arrays are stretches of DNA ranging from 25 to 60 base pairs (2000-2500 times shorter than BAC probes), designed to cover the genome of the target sample. They exist in different formats, off the shelf or custom made supports. Oligonucleotides are manufactured *in situ* by robotic automated factories using photolithography techniques adapted from the microelectronics industry.[47]

OligoCGH has been widely applied in the postnatal and prenatal setting because although individual oligonucleotide probes have a low signal-to-noise ratio and a less specific hybridization than BAC probes, the higher number of probes allows superior reliability and resolution, far superior to the threshold of 400 kb that international guides recommend and impossible to achieve with BAC-based array technology. Arrays based on oligonucleotides allow a better description of the breakpoints all over the genome with a better delineation of the genes involved (this kind of CNVs are frequently inherited, allowing an easier interpretation of their significance by the observation of the parents' phenotype).

Manufacturers have a very extensive collection of oligonucleotides and individuals, and companies can decide which regions are investigated by the array purchased by selecting the appropriate oligonucleotide. This has allowed a rapid evolution of this type of array and the existence of many specialized platforms (for example, arrays designed to investigate a single chromosome with high resolution, etc.). The recommended amount of DNA allowing prenatal analysis on fresh non-cultured amniotic cells varies depending on the platform used, from 200 ng to 2000 ng; an average amount of DNA (500 ng) has been shown to give good array results.[45]



Peng & van den Veyver. Expert Review of Obstetrics & Gynecology. 2009;4(1):81-92

Figure 4*:* BAC array vs oligonucleotide array

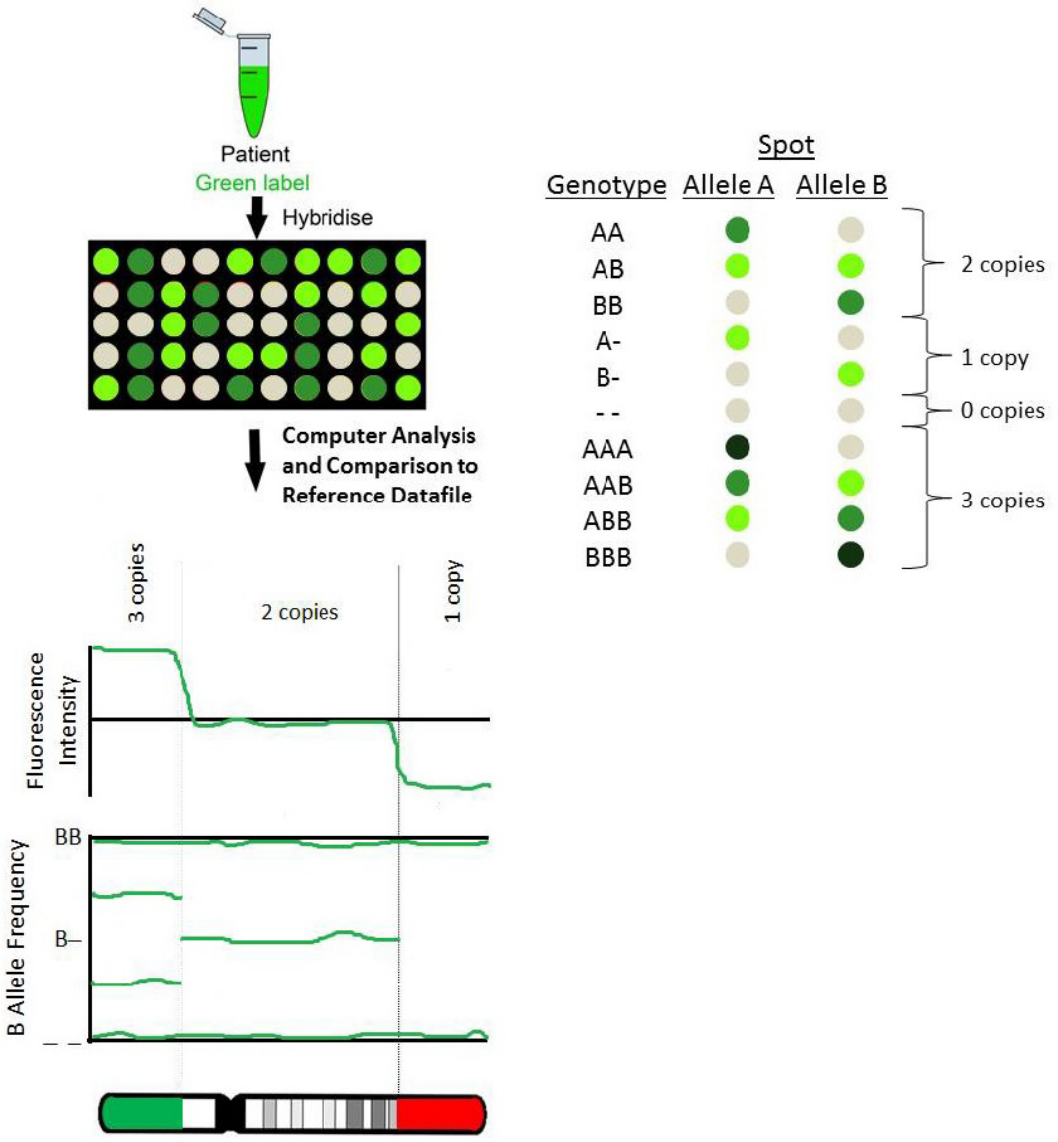
Increasing the resolution improves the detection of smaller CNVs, resulting in a higher diagnostic yield of oligonucleotide-based arrays (oligo CGH and SNP) compared to BAC arrays; a secondary effect of this is the increase of variants of unknown significance.

1.4 Single Nucleotide Polymorphism arrays

Single Nucleotide Polymorphism (SNPs) are the most common type of genetic variation. Each SNP represents a difference in a single DNA nucleotide. For example, an SNP may replace the nucleotide cytosine (C) with the nucleotide thymine (T) in a certain stretch of DNA. SNPs have been extensively used in studies examining the association of specific

SNPs with diseases. SNP arrays use pairs of oligonucleotide probes representing the two alleles of an SNP and allow testing thousands of SNPs in a single experiment

Single Nucleotide Polymorphism were developed for studies examining the association of specific SNPs with diseases and later expanded to CNV detection because fluorescence probe intensity is proportional to the number of copies of a particular stretch of DNA. SNP arrays use oligonucleotide probes of 25 bp or 50 bp long, therefore, they tend to have the lowest signal-to-noise ratio compared to other platforms, and because of this, they are less precise. Another problem is that this type of array relay on the presence of SNPs, inversely proportional to the importance of the region analysed (very critical regions do not tolerate variation). In recent years, this problem has been partially overcome by substituting an increasing number of SNP probes with conventional probes. They also can be designed with different numbers of probes covering different parts of the chromosome and higher resolution in targeted areas. Because two probes cover every SNP and their lower performance requires a greater number of probes to detect a CNV (minimum 10 probes instead of three), SNPs arrays tend to have higher probe density than oligoCGH and BAC arrays.



Karampetsou E, Morrogh D, Chitty L. J Cllin Med 2014;3(2):663–78.

Figure 5: Single Nucleotide Polymorphism (SNP) arrays

SNP microarray analysis is developed using high-density oligonucleotide-based arrays where target probes are taken from DNA locations that vary on a single base pair between individuals. Fetal DNA is hybridized then to the SNP array. The fluorescence probe intensity of the patient (fetal) samples is compared with the intensity of multiple

normal controls that were hybridized (Figure 5).[22] Recommended starting amount of material is 200-250 ng of DNA.

As CGH array, SNP arrays can detect CNVs, but more clinical information can be extracted from a genotype plot generated from the SNP array; they can detect stretches of homozygosity, allowing detection of consanguinity, parent of origin and some cases of uniparental disomy or inheritance of genetics regions only from one single progenitor (however, SNPs arrays only detects inheritance material derived from the same chromosome, but not of the two homologous chromosomes of the same progenitor) .[48] SNPs array can detect changes in SNPs distribution typically seen in somatic cancer cell changes, maternal cell contamination, and polyploidy (in this case, it would be impossible to detect triploidy with array-CGH, but this is identified by assessing the SNP allele patterns on the array).[22] SNP arrays also detect somatic mosaicism (a condition where two or more cell lines genetically different coexist in a single individual) with greater sensitivity than CGH arrays.

Table 1 summarizes the main differences between karyotype, BAC-array, oligo-array and SNP-array.

Table 1: Comparison between karyotype, Bacterial Artificial Chromosome (BAC) array, Oligo Comparative Genomic Hybridization (CGH), and Single Nucleotide Polymorphism (SNP) array

Array Platform				
	Karyotype	BAC	OligoCGH	SNP
Resolution	5-10 Mb	0.5-1 Mb	0.05-0.4 Mb	0.05-0.4 Mb
Diagnostic yield (common aneuploidies excluded)	Around 5%	2x compared with karyotype	Higher than BAC arrays	Higher than BAC arrays
Detection of VUS	+	+	++	++
Detection of CNV reduced penetrance	-	+	+	+
Starting material (ng)		50	1000 (200-2000)	200-250
Time for results (days)	6-10	3	4	4-7
Detection of MCC	Only if the fetus is male	-	-	+
Detection of triploidy	+	-	-	+
Detection of LOH/UPIC	-	-	-	+
Detection of mosaicism	+	Depends on the size of the locus, type of aberration, array platform		

VUS=variants of unknown significance, CNV=Copy Number Variant, MCC=maternal cell contamination, LOH=loss of heterozygosity, UPIC=UniParental Isodisomy

Adapted from: Karampetsou E, Morrogh D, Chitty L. J Cllin Med 2014;3(2):663–78

1.5 ARRAY DESIGN

Arrays can be "targeted" or cover the whole genome; targeted CMA brings the advantage to lower the chance of identifying VUS since the targets are associated with known phenotypes. However, if compared with whole-genome CMA, targeted CMA result in a lower diagnostic yield as genomic events that could be clinically significant could be missed since the majority of pathogenic CNVs are non-recurrent and thus, may be missed.

Whole-genome arrays use a set-up with probes covering the entire genome, overcoming the problem of missing some large relevant CNVs seen in target array design. All the probes are usually spaced in equal intervals with the problem that probes in very variable polymorphic regions are wasted and perhaps there is not enough probes in important regions.

Currently, mixed design arrays are used, with a backbone of probes that covers the entire genome and a higher density of probes in some genome regions or genes known to be of clinical importance that has been selected from an international consortium called formerly *International Collaboration for Clinical Genomics* (ICCG) and now called ClinGen.[49,50] Some array designs reach the exonic resolution in some genes of special relevance also selected by the consortium. This kind of designs are constantly under review and updated from time to time depending on the new evidence published and commercial issues.

1.6 CHROMOSOMAL MICROARRAY IN PRENATAL DIAGNOSIS

The main advantage of CMA technology over the classic cytogenetic techniques lies in its ability to detect smaller genetic imbalances. Conventional karyotype using G banding sequencing allows detecting genetic changes that are around 5-10 Mb in size[17]. Moreover, its resolution depends on the exact location of the genome analyzed, the quality of the material and the cytogeneticist's experience.[1]

Fluorescence in situ hybridization (FISH) probes for microduplication and deletion are 100-200 Kb in size but require clinical guidance to select probes. Conventional cytogenetics and FISH experiments are time-consuming and the use of multiple FISH experiments is expensive.

The implementation of chromosomal microarray offers the advantage of detecting submicroscopic genetic imbalances below the limit of 5 Mb found with conventional genetic studies and permits the study of imbalances dispersed throughout the entire genome in a single study.

Chromosomal microarray was firstly implemented in postnatal settings and pediatric population, where it dramatically improved diagnostic yield, allowed identification of new syndromes and helped the understanding of the phenotypic effect of the presence of copy number variation in the human genome and rapidly became the first-line test performed in postnatal setting in case of neurodevelopmental disabilities and congenital anomalies.[51]

For these reasons, in the last few years, the use of CMA was also validated in the prenatal setting, thus becoming one of the most useful tools for genetic diagnosis.

Several large studies have analyzed the performance of CMA compared to standard karyotype in prenatal samples with different indications: fetuses presenting abnormal ultrasound scans, advanced maternal age, abnormal serum screening result, parental anxiety and personal or familiar history of chromosome abnormalities.[52] [53] [54] Consistent evidence has arisen demonstrating that CMA presents very similar performance compared to standard karyotype for prenatal diagnosis of common aneuploidies. Moreover presents a very superior performance in the detection of submicroscopic genetic imbalances. [1]

1.6.1 CMA IN FETUSES WITHOUT ULTRASOUND ANOMALIES

Nowadays, it is standard practice to recommend CMA in those pregnancies presenting ultrasound anomalies. However, not all centers offer microarray in all pregnancies with an indication of invasive testing. So far, an association between maternal age and the prevalence of submicroscopic genetic imbalances has not been demonstrated.

A recent meta-analysis assessed the performance of CMA over karyotype in pregnancies without an ultrasound anomaly who underwent an invasive test for advanced maternal age and/or parental anxiety. The study found a pathogenic clinically significant CNV in 0.86% of cases (1:116) in which the karyotype was normal. In 0.34% of these cases with a normal karyotype, the CNV found was associated with early-onset diseases associated with developmental and intellectual disability not detectable with a prenatal ultrasound scan.[55] The author postulates that a significant number of these conditions are more severe than Down Syndrome. The difference in frequency of CNVs between tests performed for advanced maternal age and parental anxiety was not statistically significant, supporting the hypothesis that the risk of having a submicroscopic genetic

anomaly is not related to maternal age. Apart from the risk of having a submicroscopic anomaly, every woman has her own risk of chromosomal abnormalities due to her age. If the risk of submicroscopic aberration is added to one of the chromosomal anomalies, the final risk would be as high as 1:180 pregnancies; pregnant women aged less than 36 years present a higher risk of submicroscopic anomalies than for Trisomy 21.[22]

Attention has been given to the fact that older women usually have same-age partners: there is no consensus whether advanced paternal age could be related to the risk of having a CNV. In some studies, paternal age has been related to molecular changes in germinal cells that could be transmitted to the descendants[56,] and it has been shown that the father's age could determine the de novo mutation rate at the moment of conception.[57] A study with a large sample size of 6.773 healthy male participants in the Netherlands showed no evidence of an association between increased prevalence of microdeletions and microduplications in the offspring and advanced paternal age. Nevertheless, the authors did not exclude the possibility that paternal age could affect a subset of CNVs and that it could be possible that rare de novo mutations at some loci present more often with advance paternal age.[58]

When fetuses without ultrasound anomalies are considered, the incremental yield of microarray over standard karyotype has shown considerable variability in literature.

A systematic review demonstrated a clinically significant finding on the array in 1.7% of cases when CMA was performed for advanced maternal age and positive first-trimester screening result,[1] whereas others report a prevalence varying from 0.4% to 2% [59]. This difference is probably due to the different array platforms and resolutions used and

laboratory policies reporting pathogenic and VUS results. Moreover, criteria have changed over time, and, with the greater and greater sharing in international databases, the number of regions associated with disease has increased, while the incidence of variants of unknown significance has decreased.

A recent meta-analysis evaluated 8 studies with 10.314 fetuses and demonstrated that a CNV related to early-onset syndromes occurred in 0.37% of gestations; a susceptibility CNV was observed in 0.3% of the cases related to late-onset disorders.[55]

1.6.2 CMA IN FETUSES WITH ULTRASOUND ANOMALIES

Different studies have evaluated the use of CMA analysis in the prenatal context; in fetuses with ultrasound anomalies, microarray provides information over karyotype in 6-7% of pregnancies with an anomaly identified by ultrasound, most frequently cardiac, renal, skeletal, urogenital, and Central Nervous System anomalies.[60] The overall greater prevalence of CNVs is found in fetuses presenting multiple organ system anomalies compared to single system anomalies.

The NICHD study is a large scale, prospective blinded study published in 2012 which reported CNVs of clinical significance in 6% of fetuses presenting an ultrasound anomaly (755 of 3822) and a normal karyotype; an array platform that also included oligonucleotides was used to maximize the detections of microdeletions and microduplications and to identify additional chromosomal imbalances. Variants of unknown significance were detected in 3.4% (130 of 3822) cases with a normal karyotype. Of these cases, 72.3% presented findings that required a second step analysis for their exact clinical relevance. Since the study started 5 years before its publication,

and databases of array results had expanded with more information, the laboratory reinterpreted their initial definitions of VUS depending on the more recent evidence; only 56 of the initial VUS would remain of unknown significance, 30 would have been reclassified as pathogenic, and 8 would be considered as benign.[60]

Another study in 2012 performed in 5000 fetuses stated a prevalence of 6.6% in those cases (n=2462) presenting ultrasound anomalies.[53] The same authors published a second study focused on specific anomalies detected by ultrasound; in the group of fetuses presenting single organ system anomalies, in 5.3%, the array analyses demonstrated CNVs rising to 7.1% if soft markers, amniotic fluid and growth anomalies were also considered. Cerebellar hypoplasia (16.7%), holoprosencephaly (15.1%), clubfeet or hands (13.6%), and skeletal anomalies (13.3%) were the single anomalies with the highest detection rates of CNVs. 82% of the findings were less than 10 Mb, making it very hard to identify by standard fetal karyotype. When cases with multiple fetal anomalies were considered for the analyses, 9.5% presented a clinical CNV, with the highest detection rate with hypoplastic left heart (26.9%), posterior fossa anomalies (22.9%), tetralogy of Fallot (20.0%); and cystic hygroma (17.1%). In 68% of cases, the CNV detected was smaller than 10 Mb.[61]

Two meta-analyses reported a detection rate of 10 % [62] and 7%[52] of microarray over karyotype, respectively. Hillman and colleagues also reported a detection rate for VUS of 2.1% if an anomaly was found on ultrasound scan, whereas the detection rate of VUS if all indications for performing a CMA were considered was lower (1.4%).[62]

Several studies have investigated the incidence of CNVs in the prenatal setting refined by the organ system involved and the number of ultrasound anomalies detected.

Donnelly and colleagues conducted a study evaluating the association of CNVs with single and multiple organ anomalies detected by ultrasound in a group of 752 fetuses with one (n=498) or more (n=254) systems involved and a normal karyotype. In 5.6% of fetuses with single organ system anomaly, a non-benign CNV was found; this frequency was not significantly higher than the one of 3.6% (p=0.4) found in the control group (invasive test performed for advanced maternal age). Nevertheless, in this group, the indication for increased nuchal translucency (>3.5 mm) of cystic hygroma was the most frequent one. If the CNVs prevalence was evaluated when nuchal alterations were isolated, the prevalence of CNVs detected was comparable to that of the control group (3.8% vs 3.6%). Once nuchal alterations were excluded from the overall analysis in the group of isolated structural anomalies, the frequency of CNVs was higher (6.7%, p=0.009) than that of the control group. In fetuses with multiple organ anomalies, the prevalence of CNV was significantly higher (13%, p<0.001) than the one found in the control group. The most frequent anomalies in which a CMA gave an abnormal result were cardiac anomalies with a prevalence of 15.6%, facial anomalies (15.2%) and thorax anomalies (15%). When only one system anomalies were considered, the highest prevalence was found in cardiac and renal systems. [63]

1.7 FETAL MALFORMATIONS

1.7.1 CARDIAC ANOMALIES

Congenital heart diseases (CHD) are the most common defect reported at birth; a CHD is diagnosed in up to 4-50 per 1000 births [64] and is estimated to occur in approximately one in 10 stillbirths.[65] Several genetic causes are associated with cardiac abnormalities: the genetic effect in the formation of a cardiopathy is a continuously growing field with new evidence arising focusing the attention on new genes that can affect the cardio genesis. Several risks factors are associated with the development of a cardiopathy and include a family history of congenital heart disease; a maternal disease or condition - such as diabetes mellitus, collagen vascular diseases, phenylketonuria, positive SS-A/SS-B antibodies, advanced maternal age (over 35 years), increased body mass index (BMI); mother's exposure to teratogens (such as lithium, alcohol or cocaine use); prenatal infection (e.g., rubella virus); pregnancy conceived by In vitro fertilization (IVF), monochorionic gestations.[66,67]

In prenatal setting, the incidence of chromosomal anomalies when a cardiopathy is diagnosed is reported to be 18-22%, being trisomy 21 (T21), trisomy 18 (T18), trisomy 13 (T13), Turner syndrome (TS) and 22q11 microdeletion syndrome (DiGeorge/velocardiofacial Syndrome) the most frequent.[68] These conditions present variable association with advanced maternal age, clearly correlated in trisomy 21, the majority of cases results from nondisjunction during meiosis.[69]

Cardiac anomalies are the most frequent diagnosis in infants with T21, and approximately half of the patients with a trisomy 21 presents a CHD.[70,71]

In fetuses diagnosed with T21, the most common cardiopathies are atrial-ventricular septal defects which are reported in up to 45% of cases, atrial septal defects (ASD) present in 35% of cases and ventricular septal defects (VSDs) that count for 26% of cases [72]; other CHDs reported to be associated with trisomy 21 include aortic arch abnormalities, Tetralogy of Fallot (TOF), transposition of great arteries (TGA) and valvular anomalies.[73]

Trisomy 18, like trisomy 13, is associated with multiple concurrent congenital anomalies. [74] Cardiac anomalies are common in fetuses with Edwards syndrome, the most frequent reported defects are patent ductus arteriosus (PDA) that is present in 77%–88% of cases, atrial septal defects (68%–76%), ventricular septal defects (76%–94%), bicuspid aortic valve (35%), and aortic coarctation (12%). Complex congenital heart disease is found in 24% of the pregnancies with trisomy 18.[75]

Fetuses with trisomy 13 usually show high rates of cardiac anomalies, being atrial septal defects, the most common anomalies found in 53%-85% of cases, followed by patent ductus arteriosus (37%-57% of fetuses), and ventricular septal defects found in 26%–42%. In one-third of fetuses with T13 complex congenital heart diseases can be observed, such as Tetralogy of Fallot, pulmonary atresia with ventricular septal defect, or atrial-ventricular septal defects. [75,76]

In fetuses diagnosed with Turner syndrome, the incidence of cardiac anomalies is reported to vary between 23% to 50%, being the most common abnormalities left-sided lesions (including bicuspid aortic valve in 12%-18% of the cases and aortic coarctation in 7%-18% of cases). Also, more complex CHD can be present in fetuses with TS, such as

anomalous pulmonary return reported in 16% of cases and hypoplastic left heart syndrome (HLHS), carrying in these cases a severe prognosis. [77,78]

The high prevalence of aortic alterations found in patients who miss only the X chromosome's short arm indicates that haploinsufficiency for some genes located in the short arm this chromosome could be the trigger factor that contributes to abnormalities in the aortic valves and aortic arch seen in subjects with Turner syndrome.[79]

DiGeorge/velocardiofacial Syndrome, also referred to as 22q11.2 deletion syndrome, presents an estimated prevalence of 1 in 3800-6500 live births. It is an autosomal dominant condition. However, the great majority of cases (up to 90%) result from a de novo mutation (deletions).[80] The phenotype is variable, but congenital heart diseases affect 81% of patients with 22q11.2 deletions syndrome, the most frequent anomalies are conotruncal defects, including truncus arteriosus, interrupted aortic arch, tetralogy of Fallot, absence of pulmonary valves, and ventricular septal defects that can be present up to 50% of the cases.[81] Associated extracardiac anomalies include thymus hypoplasia, cleft palate, facial anomalies, and endocrinological disorders: hypoparathyroidism and hypocalcemia.[82]

Apart from these commons chromosomal alterations, fetuses with a congenital heart disease carry an additional risk of genetic imbalances due to microdeletion or microduplications resulting in specifics syndromes which include, but are not limited to, cri du chat (5p deletion), Rubinstein-Taybi (16p13.3 deletion), Wolf-Hirschhorn

Table 2: Genetic conditions associated with cardiac abnormalities.

Genetic Abnormality	Type of CHD	Affected by CHD %	Gene association
T21	ASD, VSD,AVCD, TOF	40-50	3rd copy of chromosome 21. unbalanced translocation
T13	ASD, PDA, VSD, pulmonary atresia with CHD	60-80	3rd copy of chromosome 13. unbalanced translocation
T18	ASD, VSD, PDA, CoA, bicuspid aortic valve, complex CHD	60-80	3rd copy of chromosome 18
Monosomy X	CoA, BAV, AS, anomalous pulmonary venous return, HLHS	23-50	Complete or partial absence of 1 X chromosome 45X
DiGeorge /velocardiofacial Syndrome	IAA type B, aortic arch anomalies, truncus arteriosus, TOF	70-75	22q11 deletion
Noonan syndrome	PS, hypertrophic CM, ASD	70-80	RAS-MAPK pathway
Alagille syndrome	PS, TOF	90	JAG1, NOTCH2
Holt-Oram syndrome	HCM	75	TBX5

AVCD: Atrioventricular canal defect; BAV: bicuspid aortic valve; CM: cardiomyopathy, CoA: coarctation of the aorta; HCM: hypertrophic cardiomyopathy; PS: pulmonic stenosis

Modified from: Hopkins MA, Dugoff L, Kuller JA, Obstet Gynecol Surv 2019 Aug;74(8):497-503.

(4p16.3 deletion) Williams-Beuren, Potocki-Lupski , Jacobsen (11q distal deletion) and Noonan Syndromes (being the latest a monogenic anomaly). [83,84]

Most of these conditions would not be diagnosed with a conventional karyotype, but that would be possible in some cases with a CMA.

A systematic review with a metanalysis of 13 studies reported an incremental yield of CMA in case of cardiac anomalies in 7.0% of cases once abnormal karyotype and 22q11 deletion were excluded. This incremental yield includes both direct diseases causing pathogenic CNV, and variants associated with incremented risk for neurodevelopmental delay. The additional prevalence of VUS was 3.4%. If the cardiac anomaly was present alone, the prevalence of pathogenic CNV was 3.4%, increasing at 9.3% if multiple system anomalies were present. [85] The yield increased by 12% if also 22q11 microdeletion was included.

Another report in the cohort of congenital heart disease and extracardiac anomalies found a higher prevalence of pathogenic CNVs than this metanalysis, with an incremented yield of 17-53% 81, including genetics imbalances associated with neurodevelopmental delay and dysmorphic features.

Another study reported a lower incremental yield of CMA over karyotype, reporting 4.2% CNV in cardiac diseases diagnosed prenatally. In this study, no difference was found if the cardiac anomaly was present alone or associated with other anomalies or between simple and complex defects. The authors did not report a significant effect of familiar history in causing a chromosomal abnormality.[86]

Literature report different yield when cardiac anomalies are considered isolated or when are associated to other defects; a study of Shaffer on 580 cases of congenital heart diseased diagnosed prenatally, the incremental yield of CMA over karyotype was 2.5% in isolated cardiac anomalies, rising as high as 11.7 % in non-isolated defects. Another work from Zhu *et al.* reported a pathogenic CNV in 8.2% of isolated cardiac defects and 28.6% in non-isolated ones. Other studies did not report a significant difference when isolated, and non-isolated cardiac heart diseases are diagnosed prenatally. Considering specific heart defects, ventricular septal defects (mainly peri membranous) present with an incremental yield of pathogenic results on the CMA ranging from 3% to 14%, being the cases associated with other extra cardiac anomalies the ones with the highest prevalence. [61,87–89] Conotruncal malformations (Tetralogy of Fallot, interrupted arch) and left ventricle outflow tract alterations also are common in the prenatal setting with a pathogenic result on the microarray.

1.7.2 CENTRAL NERVOUS SYSTEM ANOMALIES

Genetic factors play an important role in causing specific CNS anomalies; some well-known chromosomal abnormalities associated with cerebral anomalies are trisomy 13, trisomy 18, Miller-Dieker lissencephaly syndrome and monogenic syndromes (such as Joubert syndrome and Holoprosencephaly type 3). However, for many cerebral anomalies, the underlying cause remains undetermined. [90]

Recent reports pointed attention to underlying copy number variants as a cause of disease in cerebral anomalies with a prevalence of pathogenic CNVs in fetuses with a normal karyotype and a cerebral anomaly varying between 3.7 % and 10.9%. [91,92]

A retrospective study on a cohort of 35 terminated fetuses with an isolated CNS anomaly reported a prevalence of pathogenic CNV in 12% of the fetuses. Furthermore, a probably pathogenic CNV in the chromosomal region 3p26.3 and 12 additional rare CNV considered VUS at the time of publication were found[93].

Among specific subgroups of cerebral anomalies, the highest rates were found in neural tube defects (50%), subependymal cysts (20%) and microcephaly (16.7%). Fetuses with mild ventriculomegaly accounted for 44.7 % of the total, but interestingly, in this group, the detection rate of chromosomal abnormalities and pathogenic CNV was only 2.8% of the total. The most common pathogenic microdeletion found in this study was 16p11.2: the gene involved, T-box transcription factor 6, is a crucial gene causing vertebral deformity in patients with 16p11.2 microdeletion syndrome.[94]

A description of specific CNS anomalies and their association with genetic conditions is provided above.

Cerebral ventriculomegaly

The etiologic cause of a ventriculomegaly varies and includes a normal variation of dimension, chromosomal abnormalities, genetic syndromes, brain anomalies, congenital infections, cerebral vascular accidents. The incidence is 0.3-1.5 per 1000 live born.[95] Even when fetal ventriculomegaly is considered isolated, it carries an associated risk of abnormal neurologic developmental outcome.[95] The risk of aneuploidy when a ventriculomegaly is diagnosed is high (9-36%) in those forms with other abnormalities associated. In contrast, in isolated cases, the risk of an underlying aneuploidy is lower (1.5%-12%). Isolated forms of ventriculomegaly have been associated with

chromosomal abnormalities – mainly trisomy 21 – in many reports.[96–98] A review from 2009 reported a risk of associate aneuploidy in 2.8% of cases. [98] Ventriculomegaly can be present in many syndromic conditions like Crouzon, Goldenhar, Gorlin, Kartagener, Meckel-Gruber, Miller-Dieker, Neu-Laxova, and Walker-Warburg syndromes. [99]

Midline- Holoprosencephaly

The incidence of holoprosencephaly is estimated to be 1 in 10000-15000 live-born; it is higher in miscarriages and stillbirths, reaching 1 in 250 cases, and indicating that the great majority of fetuses carrying this anomaly end in a pregnancy loss.[100,101] The etiology is heterogeneous, and identified causes include chromosomal anomalies, teratogenic drugs, and metabolic diseases (maternal mellitus diabetes). Chromosomal abnormalities are found in 14%-47% of the cases, with trisomy 13 being the most representative aneuploidy in the case of holoprosencephaly.

Holoprosencephaly has been associated with monogenic causes, including many autosomal dominant conditions with variable phenotypic expression penetrance. Nine genes are known to be causative of holoprosencephaly in humans if mutated: *SHH*, *PTCH, GLI2, ZIC2, TDGF1, TMEM1, TGIF, FAST1* and *SIX3*. [101]

Protein *SHH* seems to have a critical role as a regulator in the ventral development of the neural tube being implicated in its induction and differentiation; a defect in the ventral induction and remodeling of the rostral neural tube has a clear role in the genesis of holoprosencephaly. Aberrations of cells of the ventral line of the neural tube that express SHH protein -or direct anomalies in the metabolic cycle of SHH protein- are critical for the correct development of prosencephalon.[102]

Nevertheless, in up to 60% of cases of holoprosencephaly, the molecular causes of the anomaly remain unknown, suggesting the implication of many genes together with environmental factors.[102]

However, the risk for a genetic syndrome is high, up to 20%-25% of cases: association with holoprosencephaly has been reported in more than 40 syndromes such as DiGeorge, Ectrodactyly-ectodermal dysplasia, Goldenhar, Meckel-Gruber, Oro-facial-digital like, and Smith-Lemli-Opitz syndrome.[103]

Midline- Corpus callosum agenesis

The prevalence of agenesis of the *corpus callosum* is estimated to be around 0.3-0.7% in the general population but increases up to 2-3% in the population with impaired neurological development.[104] The risk of chromosomal abnormality once a *corpus callosum* alteration is diagnosed is relatively high (up to 20% of cases), trisomy 18, trisomy 13 and mosaic trisomy 8 being the most frequent aneuploidies.

Both isolated complete or partial corpus callosum agenesis are associated with a risk for aneuploidy (4.8% and 7.5%, respectively).[105] Deletions and duplications are also described in association with corpus callosum anomalies: del4p16, del6q23, delXp22, dup8p21p23, dup11q23qter above others. [100]

A recent metanalysis reported a rate of chromosomal anomalies of 4.81 % (in case of complete isolated corpus callosum agenesis) and 7.45% for those cases with isolated partial agenesis. The study also reported the incremental yield of CNVs in fetuses with a corpus callosum anomaly and normal karyotype (5.74%).[106]

Posterior Fossa- Dandy-Walker Malformation

The incidence of Dandy-Walker Malformation (DWM) is estimated to be 1 in 30000 newborns. The associated risk of aneuploidy (mostly trisomy 18 and 13) is high, up to 30-35% of the cases. Also, the syndromic risk is relatively high; DWM can be found in different autosomal dominant and recessive genetic conditions such as Aicardi, Meckel-Gruber, Smith-Lemli-Opitz, Neu-Laxova, Oro-facio-digital type 1, and Walker-Warburg syndromes, among others.[100]

Moreover, a mutation in six genes (*ZIC1, ZIC4, FOXC1, FGF17, LAMC1*, and *NID1*) has been observed in some cases of Dandy-Walker malformation. These genes are thought to play a significant role in the interaction between the cerebellum and posterior fossa mesenchyme, and their mutation would disrupt this process. [107]

Posterior Fossa- Cerebellar Vermis hypoplasia

The exact incidence of vermian hypoplasia is unknown since the anomaly is often misdiagnosed prenatally as a Dandy-Walker variant, a Blake pouch cyst, or a mega cisterna magna. Moreover, in past literature, the terms hypoplasia and agenesis have been interchanged. It is often present as part of Mendelian syndromes, with a poor prognosis; oligophrenin-1 gene mutations have been described in male subjects with X-linked cerebellar vermis hypoplasia. [108]

Posterior Fossa- Mega Cisterna Magna

Mega cisterna magna is a relatively frequent diagnosis in the prenatal setting, with a prevalence of around 0.8%.[100] In the prenatal setting, it is typically an incidental finding,

and many authors consider it a normal variant; its association with aneuploidies and syndromic conditions is low. In a recent metanalysis, none of the fetuses tested prenatally was found to have a chromosomal abnormality.[109]

Posterior Fossa- Blake's pouch cyst

Blake's pouch development is a normal phase of development of the structures of the posterior fossa. When there is a failure or delay of fenestration of the embryonic Blake's pouch, there is a failure in the communication between the fourth ventricle and the subarachnoid space, and cystic formation appears; it has the same radiographic appearance as arachnoid cysts and has an unknown prevalence.

The risk of chromosomal abnormalities based on the limited evidence present in literature is low.[100]

Posterior Fossa- Cerebellar hypoplasia

Cerebellar hypoplasia refers to abnormal development of the cerebellum. Cerebellar hypoplasia presents a wide etiological spectrum, including both primary (malformities) and secondary (disruptive) conditions. In literature, it is reported to be associated with different chromosomal abnormalities, being trisomy 13 and 18 the most frequent aneuploidies described. [110] Other less consistent associations are described in the literature, such as with Trisomy 21 (complete or mosaic), trisomy 17 mosaicism, monosomy 1p36, translocations, and sex chromosomal anomalies (X monosomy). [111]

Among the primary conditions also genetic syndromes are described, such as Ritscher-Schinzel, Joubert, and CHARGE syndromes. [112]

Posterior Fossa- Romboencephalosynapsis

This rare malformation usually presents with a sporadic nature and a low recurrence risk. Most cases are non-syndromic, however, it is frequently found in Gómez-López-Hernández syndrome[113] and in fetuses with VACTERL association (Vertebral anomalies, Anal atresia, Cardiovascular anomalies, TracheoEsophageal fistula, Renal anomalies, and Limb defects). [114]

Neural Tube defects

The term includes different conditions resulting from a defect in the closure of the neural tube. The most frequent are spina bifida (prevalence of 1 in 1000 new-borns), encephalocele (prevalence of 0.3-2/10000 new-borns) and acrania (prevalence of 1/1000 new-borns but is diminishing as a result of early prenatal diagnosis).[100] Acrania has a low risk of both aneuploidy (1-5%) and associated syndromes. Encephalocele is associated with a relatively low risk of aneuploidies of 4-9%, but different syndromes are described as being associated like Meckel-Gruber, Mohr, Roberts, Walker-Warburg, and Chiari III syndromes. Spina bifida has a relatively high risk of aneuploidies (between 8 and 14%) but a low syndromic risk. Fewer than 10% of all neural tube defects are syndromic and can occur in chromosomal disorders (including T13 or T18), but the majority are non-syndromic with a sporadic pattern of occurrence.[115]

In studies based on animal models, many genes implicated in the causation of neural tube defects (NTD) have been found, and some are highly conserved evolutionarily, with a role in the neurulation demonstrated in multiple vertebrate models.[116] In humans, genetic variants have been found associated with an increased risk of having a neural

tube defect. However, some of these variants are found both in patients with an NTD and healthy controls, making a clear correlation genotype/phenotype challenging to demonstrate. [117]

1.7.3 FACIAL ANOMALIES

During the embryologic phase, the correct fusion between the facial processes depends on different events involving cell migration, growth, adhesion, differentiation, and cellular apoptosis. Disruptions in the fusion of the facial processes may result in complete or partial clefts of the face, lip and/or palate.

Anophthalmia and microphthalmia

These conditions can be present unilaterally or bilaterally. The prevalence at birth of anophthalmia and microphthalmia is reported to be 1 per 10000 new-borns.[118] In most cases, the diagnosis of these severe conditions is made because of the presence of other fetal abnormalities being the orbital finding a part of a syndrome; only very rarely orbital alterations are diagnosed prenatally as isolated findings. The risk of associated aneuploidies and syndromes is extremely high.

Microphthalmia is one of the findings usually associated with trisomy 13, being present in more than 50% of the cases of T13. Also, triploidy and mosaic trisomy 9 are among the aneuploidies most likely to feature microphthalmia.

Syndromes that have been reported with associations with eye anomalies include Aicardi, Fraser, Fryns, Goldenhar, Gorlin, Lenz and Walker-Warburg syndrome. [119]

Hypotelorism

Hypotelorism is a rare condition, rarely is an isolated finding, being associated most with holoprosencephaly spectrum and craniosynostosis. For its association with holoprosencephaly, hypotelorism has a high risk of aneuploidy and is related electively with trisomy 13. The syndromic risk also is very high, an association with midline brain defects, such as septo-optic dysplasia and Cruzon syndrome, is reported.[120]

Hypertelorism

As hypotelorism, hypertelorism is a rare condition usually associated with many different syndromes. Chromosomal abnormalities that feature hypertelorism include 4p deletion (Wolf-Hirschhorn syndrome), 9p duplication, tetrasomy 12p (Pallister-Killian syndrome), triploidy and trisomy 18. Some cases of trisomy 13 also are described as associated. The syndromic risk is very high. Syndromes that are associated with hypertelorism include Noonan, campomelic dysplasia, chondrodysplasia punctata, Larsen, multiple pterygium syndrome, Roberts syndrome, craniosynostosis syndromes (Apert, Crouzon, and Pfeiffer), Pena Shokeir, Opitz BBB, syndromes and CHARGE association (Coloboma, Heart defects, Atresia choanae growth Restriction, Genital anomalies, and Ear anomalies). [119,121]

Orofacial Cleft

Orofacial clefts present a prevalence of 1 in 700 births. Most clefts are paramedian: 64% are unilateral, and 34% are bilateral. [122] The risk of chromosomal abnormalities is greater in associated lip-palate cleft vs only lip cleft and bilateral forms vs unilateral forms. The median cleft is the variant with a worse prognosis because it is often

associated with holoprosencephaly or other severe midline anomalies that carry a greater risk of genetic abnormalities. [100] The majority of the paramedian orofacial clefts are not associated with common aneuploidies, and if the cleft is isolated, identifying an underlying genetic cause is less likely; however, lip-palate cleft has been associated with more than 400 syndromic conditions. Median clefts are less frequent, and are estimated to account for approximately 0.38- 3% of all orofacial clefts. [123,124] Several chromosomal and genetic syndromes have been associated with a median cleft; primary craniofacial syndromes associated with a median facial cleft are typically within the holoprosencephaly spectrum and are often secondary to aneuploidy (especially trisomy 13). Syndromic conditions frequently associated with orofacial clefts are Goldenhar, Fraser, Ectrodactyly-Ectodermal dysplasia, frontonasal dysplasia and Fryns syndrome.[125–127] CNVs detected with CMA are reported in up to 10% of cases of orofacial clefts, including 22q11.2 microdeletion. [61]

Micrognathia

Micrognathia and retrognathia refer to an abnormal mandible, the first a hypoplastic mandible, and the second is a mandible displaced posteriorly concerning the maxillary bone. Micrognathia is virtually never isolated, being present in a high number of different syndromic conditions. Also, in those cases thought in utero to be isolated, it is very common to find postnatally associated anomalies and syndromes.[128] is a common feature of many aneuploidies, especially trisomy 13, trisomy 18 (up to 70% of cased with T18 present a variable degree of micrognathia at post-mortem exam) and trisomy 9. [100] Syndromes that include micrognathia are numerous: primary mandibular syndromes include Pierre Robin, Nager, Treacher Collins and orofacial digital syndromes;

skeletal and muscular disorders associated include: skeletal dysplasia, craniosynostosis syndromes; other syndromes include: DiGeorge, Smith-Lemli-Opitz syndrome, Goldenhar syndrome, Noonan syndrome, Meckel-Gruber syndrome, Fryns syndrome, Pena-Shokeir syndrome and Joubert syndrome. [129]

Nasal abnormalities

A group of rare abnormalities that include proboscis, arrhinia, and cebocephaly are all part of the spectrum of midline anomalies. They carry an extremely high risk of chromosomal abnormalities and are electively associated with trisomy 13 and holoprosencephaly. Minor anomalies of the nasal aspect are rare too and carry a lower risk of chromosomal abnormalities (especially trisomy 21 in cases of nasal bone hypoplasia-agenesis), and the syndromic risk is variable. [100]

External ear deformities

Microtia, macrotia, and ear tags are the external ear anomalies that can be easier recognized in utero, and that can be a syndromic prognostics factor if associated with other abnormalities (especially the presence of preauricular ear tags). The risk of chromosomal anomaly is high, especially for Trisomy 13 and trisomy 18. The syndromic risk is also high, excluding those conditions in which only low ear implantation occurs. Some syndromes present with a significant external ear anomaly (e.g., Goldenhar, Fraser, Nager, Treacher-Collins syndromes).

1.7.4 CONGENITAL THORACIC MALFORMATIONS

Congenital thoracic malformations account for 5–18% of all congenital abnormalities, presenting an incidence of 30–42 cases per 100.000 individuals.[130]

Congenital diaphragmatic hernia

Congenital Diaphragmatic Hernia (CDH) is a rare condition with a prevalence of 1 to 4 per 10,000 pregnancies[131]; 75-85% of CDH are left sided (Bochdalek variant), 10-15% are found in the right hemidiaphragm, and 3-4% are bilateral. CDH present a low risk of aneuploidy (5-15%, being trisomy 21 and 18 the most common aneuploidies associated); CDH is also observed in some chromosomal anomalies like 9p tetrasomy, and it is often found in association with syndromes like Fryns syndrome, Pallister-Killian syndrome, Beckwith-Wiedemann syndrome. [100,132,133] Recent studies using CMA reported 9% of cases to have clinically relevant copy number variants[134]; moreover, different monogenic conditions present a CDH among other abnormalities in their phenotype. [135]

Congenital pulmonary airway malformation

Congenital pulmonary airway malformation (CPAM) presents a low risk of chromosomal abnormalities (aneuploidies are a rare finding, and when an aneuploidy is found, there is usually an associated extra-pulmonary anomaly). Also, the syndromic risk is low.

Pulmonary sequestration

Pulmonary sequestration presents a prevalence between 0.15 and 1.8% [136]; as CPAM, it presents a low risk of both aneuploidies and syndromic conditions.

Congenital High Airway Obstruction Syndrome

Congenital High Airway Obstruction Syndrome (CHAOS) comprehends a series of rare anomalies, including laryngeal and tracheal atresia. These conditions have a low risk of aneuploidy but a remarkably high syndromic risk. A significant part of tracheal and

laryngeal atresia cases is associated with Fraser syndrome, an autosomal recessive condition associated with laryngeal atresia, facial cleft, cardiopathies, and microphthalmia, ambiguous genitalia, bilateral renal agenesis, and ear anomalies. [137]

Hydrothorax

The incidence of primary fetal hydrothorax is estimated to be 1 in 15,000. [138] Hydrothorax has a remarkable association with aneuploidies, also in those cases in which the anomaly is present as isolated and transitorily, and it is associated with Trisomy 21 and Turner Syndrome. In fetuses with second and third trimester pleural effusions, the reported aneuploidy rate ranges between 3.2% and 5.8%. [139] Also, chylothorax is found to have an association with aneuploidy as high as 1.6% of the cases. [140]

1.7.5 GASTROINTESTINAL ANOMALIES

Esophageal atresia

Esophageal atresia is a relatively frequent condition with a reported prevalence of 2.3 per 10.000 live births. [141] In most subtypes of esophageal atresia, a tracheoesophageal fistula is found. In up to 50% of cases, additional structural anomalies are present, as a part of a genetic condition or association, such as the VACTERL association. [142]

The risk of chromosomal anomalies is high. In prenatal diagnosis, the risk of aneuploidy is reported to be 20-44%, with a higher prevalence for trisomy 21 and 18. [100] Other chromosomal structural and numerical imbalances have been reported, like trisomy 13, 22q11 deletion syndrome, 13qdel or 17qdel, and pathogenic variants in single genes (*MYCN, CHD7, SOX2, FANCB*). [143]

Duodenal atresia

The incidence of duodenal atresia is reported to be approximately 1 in 7500 births, with a high frequency of associated chromosomal abnormalities, particularly trisomy 21 (it is reported a range of associated Down syndrome in 30-50% of the cases of duodenal atresia). In postnatal series 3-5 % of newborns with trisomy 21 present with duodenal atresia. [144,145] While the anomaly is mostly associated with Down syndrome recent studies report additional genetic associations of duodenal atresia, including 4q22.3 microdeletion and heterotaxic syndrome. [146,147]

Exomphalos

Exomphalos is a common abdominal wall defect, with a reported frequency of 1 in 5,000 gestations, and a reported incidence of 0.8 in 10,000 live births.[148] This difference between pre and postnatal is due to the high frequency of associated anomalies and chromosomal abnormalities which occur in approximately 40-50% of cases, and often result in stillbirth or termination of pregnancies. [149] The most common aneuploidies associated with exomphalos are trisomy 18, trisomy 13, and triploidy (the risk is higher in those cases without liver herniation). Also, the syndromic risk is high: Beckwith–Wiedemann syndrome in association with omphalocele is reported in 8-10 % of the cases. [150,151]

Gastroschisis

Gastroschisis affects 1 in 2,000-5,000 gestations with an increasing prevalence reported over the past two decades. [152] Usually the defect is isolated and is not associated with chromosomal abnormalities; it is reported that only 1.2% of new-borns with

gastroschisis have an abnormal karyotype; in this group the most frequent aneuploidies are trisomy 18, trisomy 13, sex chromosome anomalies and trisomy 21. The association with genetic syndromes is low, it is reported to be around 2%. [153]

Anorectal malformations

Anorectal malformations affect approximately 1 in 2,500 to 1 in 5,000 new-borns. [154] The terms include different degrees of severity, ranging from mild anal stenosis over anal atresia to cloacal anomalies. [155] The risk for aneuploidy is high, especially for trisomy 21 and trisomy 18. The syndromic risk also is high; syndromes most frequently associated with anorectal malformations are VACTERL association, sirenomelia and caudal regression syndrome. Genome-wide CNVs screening found that 13q deletions and a de novo microduplication at 22q11.21 may be implicated in syndromic patients with anorectal malformations. [156,157]

1.7.6 UROLOGIC ANOMALIES

Anomalies of the genitourinary system are among the commonest anomalies identified prenatally, with a reported incidence of 1 to 4 in 1,000 gestations. [158]

Congenital urological anomalies present a not well-defined pathogenesis; many cases are sporadic, but family clusters are common, suggesting a genetic role in the phenotype. It is reported that up to 20% of patients may present an underline genetic disorder, which is not detected with standard clinical evaluation.

Over 40 genomic disorders have been described implicated in both syndromic and non-syndromic forma of urological congenital anomalies. [159]

Renal agenesis

Renal agenesis can be present unilaterally or bilaterally, with an incidence of 1 in 1,000 and 1-2 in 5,000 gestations, respectively. The risk for aneuploidy is low in unilateral forms being below 1%; the risk rises slightly in bilateral forms with a reported incidence of 1-2%. Conversely, the syndromic risk is higher, up to 20-25% of cases; the most frequent associations with syndromes are with Fraser syndrome, VACTERL association, caudal-regression syndrome, sirenomelia, Oro-cranio-digital syndrome [160]

Renal ectopia

The term includes different conditions in which an anomaly in the renal position is present: pelvis kidney and horseshoe kidney. Pelvic kidney has a low risk for chromosomal abnormalities, whereas for the latter the risk of aneuploidy is 5-8% (mostly trisomy 18 and Turner syndrome), and the syndromic risk is reported to be 15-20%. Horseshoe kidney is also reported in the following conditions: Acro-renal Dieker type, acro-renal Siegler type, caudal-regression syndrome and MURCS association (Müllerian duct aplasia, renal aplasia, and cervicothoracic somite dysplasia). [160]

Polycystic kidney disease

The term refers to different conditions that can be present in utero or remain silent until adulthood. Autosomal dominant polycystic kidney disease (ADPKD) is the most frequent inherited renal condition (prevalence of 1 in 1000) with a mutation in two genes of chromosome 16, *PKD1* (responsible for 85% of cases) and *PKD2* (that account for another 10-12%). A third gene is suspected to be involved in the remaining cases but the evidence is still limited. [161] Autosomal recessive polycystic kidney disease (ARPKD) is a

rare condition, described in approximately in one in 20,000 livebirths and it is caused by a mutation in a gene which is located on chromosome 6p, PKHD1. Since it is a monogenic disorder, the karyotype is not helpful, whereas the structural characteristics of these kidneys can be found also in other syndromes, such as Bardet-Biedl and Meckel Gruber. [162]

Dysplastic kidney

Dysplastic kidneys can be any size, ranging between massive kidneys with multiple large cysts up to 9 cm in diameter (commonly named multicystic dysplastic kidneys (MCDK)), to normal or small kidneys, with or without cysts.

Multicystic dysplastic kidney (MCDK) is a common subset of renal dysplasia with an incidence of 1 in 3,640 live births. [163] It is more prevalent in the unilateral form and is typically sporadic, although familiar cases are reported. The anomaly can be isolated, associated with other genitourinary abnormalities, or as part of a genetic syndrome (the most frequent associated syndromes are Brachial-oto-renal syndrome and VACTERL association).

The risk of aneuploidy is low in the isolated forms (2-4%), reaching a higher prevalence in the bilateral and not isolated forms (with a reported risk of 15-18% and 25-28% respectively). [164] A bilateral dysplasia can be associated with aneuploidy or a genetic condition in a higher proportion of cases, being present in Bardet-Biedl syndrome, VACTERL association, Schinzel-Giedion syndrome, branchio-oto-renal dysplasia, and Meckel Gruber syndrome. [165,166]

Hydronephrosis

Fetal hydronephrosis is usually a clinical expression of an obstructive anomaly of the urinary tract, it can be associated with ureteral dilation. The prevalence is 1 to 5 in 5,000 new-borns and it represents around 50% of renal anomalies diagnosed in the prenatal setting. [167] In isolated forms the risk of aneuploidy is low, and the syndromic risk is low too, (around 6-8%). Syndromes reported with more frequency are Campomelic Dysplasia, Schinzel-Giedion syndrome, and VACTERL association.

Megacystis

The most frequent cause of megacysts is a bladder outlet obstruction, known as lower urinary tract obstruction (LUTO); besides those cases associated with obstruction, different causes are described, including chromosomal abnormalities, genetic syndromes, and developmental anomalies. The risk of aneuploidy is around 8-23%, with a predominance of trisomy 18, trisomy 21, Turner syndrome, and Trisomy 13. Megacystis can be present as a part of syndromic conditions such as: Prune-Belly syndrome, Megacystic microcolon intestinal hypoperistalsis syndrome, Fraser and Smith–Lemli–Opitz syndromes among others. [168]

1.7.7 GENITAL ANOMALIES

Ambiguous genitalia

The term is used when external genitalia is different from the genetic sex or when it is not possible to differentiate between male and female phenotype. The reported incidence is 1 in 50,000 new-borns; [100] however, in a recent report using Not Invasive Prenatal Test (NIPT) , a higher prevalence of sex discordant results is described, up to 1

in 1,500 to 2,000 gestations.[169] For the diagnosis, it is usually complicated to differentiate between micropenis with cryptorchidism and clitoris anomalies. In male subjects, it is frequent to diagnose micropenis, hypospadias and scrotum bifidum. In female subjects, it is frequent to find hypertrophic clitoris. [170]

In most cases the final diagnosis can only be made only postnatally. For ambiguous genitalia the reported risk of aneuploidy is low, but different chromosomal abnormalities are reported being associated: Trisomy 13, triploidy, 13q syndrome, Xp21 duplication, 9p23 deletion, 10q26 deletion. The syndromic risk is high; some of the known syndrome are Smith-Lemli-Opitz syndrome, CHARGE, campomelic dysplasia. [100]

1.7.8 SKELETAL ANOMALIES

Skeletal dysplasia

Skeletal dysplasias are disorders characterized by abnormal development of bones and cartilage. Skeletal dysplasias are generally anomalies of the skeleton, whereas dysostoses are disorders with a single or group of abnormal bones.

There are 461 different dysplasias classified into 42 groups following a classification from 2019. [171] They can be transmitted as autosomal dominant, recessive, or X-linked disorders; some can also result from somatic mosaicism, teratogens, or imprinting errors. [172,173] In the last years, 437 genes have been related to skeletal dysplasias, although the genetic defect remains unknown in 8% of the cases.[171]

An association with aneuploidies is reported in 18 -20 % of the cases in some series using cytogenetic studies. [174]

Polydactyly

Polydactyly refers to those conditions in which an extra digit is present, with or without bone tissue. It can be postaxial (ulnar or fibular side of an extremity), preaxial (radial or tibial side of the extremity), or central. Most cases are considered isolated conditions, although more than 100 genetic conditions are described presenting an association with polydactyly. [175]

Some of the chromosomal abnormalities that are associated with more frequency with polydactyly are aneuploidies, such as trisomy 13, and syndromes such as: Meckel-Gruber syndrome, Smith-Lemli-Opitz syndrome, Carpenter syndrome, Pallister-Hall and Greig cephalopolysyndactyly syndromes. [176–179] Most of these cases present an autosomal recessive inheritance and a recurrence risk of 25%, with the exclusion of Greig cephalopolysyndactyly and Pallister-Hall syndromes, which present an autosomal dominant inheritance. [176–178,180]

Congenital talipes equinovarus

Congenital talipes equinovarus (also called clubfoot) is one of the most common malformations when the skeletal system is considered. It is reported in 1 to 3 per 1000 live births and occurs more often in male fetuses (twice than female fetuses). [181] It can be unilateral (in 30-40% of the cases) or bilateral (60-70% of the cases). In 50-70 % of the cases, it presents as an isolated malformation; in the remaining 30-50% of cases is associated with structural or genetic anomalies.[182] Among the genetic causes, chromosomal abnormalities are described in up to 30% of complex cases and 2% of isolated cases, being trisomy 18, 13 and 21 the most frequent aneuploidies and 4p, 18q

and 22q11.2 the most frequent deletion syndromes.[61,183]Some genetic syndromes are also associated with clubfoot: Larsen, Gordon, Pierre- Robin, Pena-Shokeir, Meckel-Gruber, Smith-Lemli-Opitz, Roberts, talipes equinovarus, atrial septal defect, Robin sequence, persistence of left superior vena cava, and Lambert syndrome, among others. Some specific skeletal dysplasias also can present with talipes, such as Ellis van Creveld syndrome, chondrodysplasia punctata, and campomelic dysplasia. [184]

1.7.9 HYDROPS FETALIS

Hydrops fetalis presents an incidence of 1 in 1700-3000 gestations. It is diagnosed by prenatal ultrasound scans when at least 2 pathologic fluid collections are present (ascites, hydrothorax, pericardial effusion, or skin edema). [185] In the past, the great majority of cases were caused by red cell alloimmunization. However, with the widespread use of Rh(D) immune globulin, the prevalence of immune hydrops has consistently decreased. Nowadays, non-immune hydrops fetalis (NIHF) accounts for almost 90% of cases of fetal hydrops [186]

A variety of genetic causes are described in non-immune hydrops fetalis, but nearly half of non-immune cases remain of unknown etiology. [187]

Chromosomal anomalies are among the possible causes of NIHF, particularly Turner syndrome and Trisomy 21 that account for 13% as reported in a large review. [188]

Turner syndrome is found in 50-80% of cases of cystic hygroma, being lymphatic dysplasia the cause of NIHF in these cases. Other aneuploidies are described in association with hydrops, including trisomy 18, trisomy 13 and triploidy. [189]

Data about the contributing role of microdeletions and duplication are still limited. Moreover, it is reported that up to 19% of all cases of NIHF could be caused by rare genetic syndromes and up to one-third of cases would be caused by lysosomal disorders, which cannot be detected by karyotype or CMA. [190]

PART 2

JUSTIFICATION OF THE STUDY

2 JUSTIFICATION OF THE STUDY

When the present study was designed, the array technology had been implemented and was under study for its applicability and its performance compared with standard cytogenetics techniques in the prenatal setting. Arrays were first offered in case of very specific structural malformation and then were progressively being introduced for more indications.

This work may help determine some genetic abnormalities diagnosed by array-CGH in specific structural abnormalities, still to be determined or object of debate. The detection of a continuously growing number of CNVs related to specific structural anomalies allows reclassifying some genetic anomalies previously considered of unknown significance as pathogenic. It permits the discovery of new genes related to diseases and to know the genetic etiology of a growing number of clinical conditions.

PART 3

HYPOTESIS

3 HYPOTHESIS

The main hypothesis of the project is that the prevalence of array CGH alterations increased in fetuses with structural abnormalities.

Other hypotheses are:

- The prevalence of array-CGH alterations is higher in fetuses with structural abnormalities in specific organs/systems.
- The prevalence of array-CGH abnormalities is higher in specific structural abnormalities.
- There might be specific patterns of CNVs in specific structural abnormalities.
- Some factors, such as associated anomalies, fetal growth restriction, may modify the prevalence of pathogenic CNVs and VUS.

PART 4

OBJECTIVES

4 OBJECTIVES

The main objective of the project is to evaluate the prevalence of array CGH alterations in fetuses with structural abnormalities.

Secondary objectives are:

1. To describe the prevalence of pathogenic CNV and VUS in fetuses with structural abnormalities in specific organs/systems.
2. To describe the prevalence of pathogenic CNV and VUS in specific subgroups of anomalies.
3. To describe if the prevalence of pathogenic CNVs and VUS changes in isolated defects or in association with other structural defects or fetal growth restriction.
4. To describe whether there are patterns of CNVs that are recurring in certain malformations or systems.

PART 5

METHODS

5 METHODS

5.1 DESIGN OF THE STUDY

This is an observational retrospective single-centre study.

5.2 SETTING

The study was performed at the Department of Maternal-Fetal Medicine in a collaborative effort with the Department of Clinical and Molecular Genetics of the Vall d'Hebron University Hospital in Barcelona, Spain, between January 2009 and December 2017.

5.3 ETHICAL APPROVAL

This study was performed in line with the principles of the Declaration of Helsinki. The Ethical approval for this study (PR(AMI) 08/2016) was provided by the *Comité de ética de investigación con medicamentos* (CEIm) from the Vall d'Hebron University Hospital, Barcelona, Spain.

5.4 STUDY POPULATION

The study population consisted of pregnant women attending the Fetal Medicine Unit of the Vall d'Hebron University Hospital. Inclusion criteria were: 1) one or more fetal structural abnormalities and 2) fetal array-CGH study. Exclusion criteria were abnormal quantitative fluorescence-polymerase chain reaction (QF-PCR) for chromosomes 21, 18 13 or sex chromosomes and fetal infections (Cytomegalovirus, toxoplasma, Zika, Herpes virus) and failure to obtain a result.

The following data was collected: maternal age, gestational age at the moment of performance of the invasive test, type of invasive test (chorionic villus sampling, amniocentesis, fetal blood, or fetal tissue biopsy), QF-PCR, array-CGH results, associated structural anomalies and the presence of an associated fetal growth restriction (FGR).

5.5 CLINICAL PROTOCOL

Following the finding of a fetal structural abnormality, the pregnant women were referred to the Fetal Medicine Unit of Vall d'Hebron University Hospital.

5.5.1 FETAL ULTRASOUND SCAN AND COUNSELLING

A detailed ultrasound was performed to 1) give a detailed description of the abnormality; 2) look for other structural abnormalities in other organs or systems; 3) assess fetal growth; 4) assess the placenta, amniotic fluid, and umbilical cord.

In CNS anomalies, a detailed fetal ultrasound and an advanced neurosonography were performed by a fetal medicine specialist following ISUOG guidelines.[191]

In cardiac anomalies and advanced fetal echocardiography was performed by a fetal medicine specialist and a pediatric cardiology specialist, following the ISUOG guidelines.[192]

Magnetic resonance imaging (MRI) was requested in selected cases. MRI studies were performed by a Pediatric Radiologist with high expertise in fetal brain imaging using a 1.5 T system (Avanto, Siemens, Erlangen, Germany) with high-speed sequences of T2 and T1-weighted (10-15 sec). HASTE (Half-Fourier Acquired Single-Shot Turbo Spin-Echo)

sequences were obtained, T2-weighted in a multiplanar fashion, and T1-weighted on the axial plane.

After the ultrasound scan, a detailed assessment of the pathology was given to the parents. The counselling was multidisciplinary, involving geneticists, neonatologists and specialists related to the pathologies such as a pediatric neurologist, pediatric cardiologist, pediatric surgeon, maxillo-facial surgeon, pediatric urologist, or pediatric ophthalmologist.

5.5.2 INVASIVE TESTING

An invasive test was offered in all cases. In pregnancies between 11 and 14 weeks, a chorion villous sampling was performed. In pregnancies 15 weeks or older, an amniocentesis was performed. Both techniques were performed following the ISUOG guidelines.[11]

In those women that declined invasive testing and opted for termination of pregnancy, a post-mortem array-CGH study from a fetal sample was offered to the parents. In all cases of invasive testing, pre and post-test genetic counselling was offered.

5.6 GENETIC COUNSELLING

From the beginning of the implementation of microarray, our hospital's policy has always been to report only pathogenic or probably pathogenic CNVs in the prenatal setting. Women were informed that they would not be informed of CNVs of benign or uncertain significance unless they stated otherwise.

Genetic Testing

Firstly, a QF-PCR for chromosomes 21, 18, 13, X and Y was conducted. The array-CGH study was then performed in all cases in which the QF-PCR was normal.

Array-CGH technique

DNA was extracted from uncultured or cultured samples of amniotic fluid and chorion biopsies using the iGENatal genomic DNA extraction Kit (igenBiotech, Madrid) and subsequently analyzed with QF-PCR Devyser Complete kit (Devyser, Sweden), following the recommendations of the manufacturers. If QF-PCR detected any aneuploidy, karyotype analysis was performed to confirm the result and discard structural alterations, otherwise fetal DNA was analyzed with CytoSure Constitutional 8 × 60K v3 (ogt, UK) or qChip Pre 8 x 60K (qgenomics, Spain) array comparative genomic hybridization assays following the recommendations of the manufacturers. Both arrays have mixed designs, with a backbone of an average resolution of 350-663 Kb and a higher resolution (of 100-375 Kb) in regions associated with pathology. Ogt arrays have an exonic resolution in 354 genes selected by the ClinGen Dosage Sensitivity Map[193]. In some cases, because low quality of the DNA sample, a custom-made low-resolution BAC array was utilized.

Array-CGH results evaluation

CNVs were classified following recommendations of the American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants (in brief, rare recessive variants not related to fetal phenotypic abnormalities and CNVs classified as benign were not reported).[194,195]

Additionally, to reduce the anxiety of pregnant couples (a written informed consent was obtained in all the cases), findings of uncertain significance and low penetrance were not reported in ongoing pregnancies . VUS is defined as a CNV in which firm conclusions regarding clinical significance are not yet established because of a lack of information or contradictory information in publications and/or databases. While this study was ongoing, some international scientific societies published similar recommendations. [194,196–199] All variants, reported or not, were included in our analysis except benign CNVs or VUS of smaller than 400 kb.

Except for some well-known recurrent structural abnormalities known to be always "de novo", parents of all fetuses with pathogenic or probably pathogenic CNVs were proposed to be investigated with karyotype, BAC, FISH or array-CGH to evaluate a possible recurrence risk. With VUS or variables of low penetrance not reported by our reporting policy, parental samples were investigated only if available or findings were communicated after the termination of pregnancy.

The results were given in a specific consultation of post-test genetic counselling, explaining the results and the implications crucial to support informed decision-making.

5.7 VARIABLES OF THE STUDY

Main Outcome

Array-CGH results:

- Benign CNVs: CNVs described in healthy controls, not coincident with known pathogenic CNVs of incomplete penetrance and not enriched in patients'

cohorts. CNVs not described in healthy controls but without significant coding regions were considered probably benign.

- Pathogenic CNVs: CNVs that are known to cause syndromes. These CNVs are described in patients with similar phenotypes and are absent or enriched in normal controls. If evidence is not absolutely definitive but is very suggestive of pathogenicity, CNV is considered probably pathogenic.

- VUS: those CNVs with uncertainty regarding their clinical significance because of insufficient or contradictory information.

Demographic Characteristics

- Categorical variables: Parity (nulliparous or multiparous), type of pregnancy (singleton or twin) and chorionicity in multiple pregnancies (dichorionic diamniotic, monochorionic diamniotic or monochorionic monoamniotic).

- Quantitative variables: maternal age (years) and gestational age at invasive testing (weeks).

Ultrasound Findings

- Isolated or associated. When the fetal malformation was limited to a single organ or system, it was classified as isolated, and if it affected more than one organ or system, it was classified as an associated anomaly.

- Fetal growth restriction: Estimated fetal weight below the 3rd percentile or under the 10th percentile with Doppler anomalies. [200]

- Organ or system. Fetal malformations were classified as affecting CNS (brain or spine), heart (cardiac), thoracic (lungs, mediastinum), gastrointestinal (liver, gallbladder, esophagus, stomach, bowel, anus, mesentery or peritoneum), face (eyes, nose, mouth, ears, superior or inferior maxilla), urinary system (kidneys, ureters, bladder, urethra), genital (penis, testes, vulvar, clitoris, vagina, uterus, ovaries), skeletal (three segments of the upper or lower extremities, spine anomalies, general bone dysplasia) or hydrops (2 or more of: ascites, hydrothorax, pericardial effusion, subcutaneous edema).

- Subclassification.

    - CNS anomalies: Ventriculomegaly (mild, moderate, severe), Neural tube defects (Acrania, Encephalocele, Spina bifida), Midline anomalies (complete agenesis of the corpus callosum, partial agenesis or dysgenesis of the corpus callosum, holoprosencephaly, cavum septum pellucidum agenesis), Posterior fossa anomalies (Dandy-Walker malformation, cerebellar hypoplasia, vermian hypoplasia, mega cisterna magna, Blake's pouch cyst, posterior fossa cyst, rhombencephalosynapsis), Cortical development anomalies (macrocephaly, microcephaly, polymicrogyria, abnormal sulcation/gyration), Hypoxic-ischemic or hemorrhagic lesion (hypoxic-ischemic lesion, brain hemorrhage, venous sinus thrombosis), Intracranial cyst (arachnoid cyst), Periventricular hyper echogenicity, Tumors.

- Cardiac anomalies: conotruncal malformations (right aortic arch, aberrant right subclavian artery, tetralogy of Fallot, aortic override, great arteries transposition, pulmonary atresia, truncus arteriosus, aorto-pulmonary window); left heart malformations [Hypoplastic Left Heart Syndrome (HLHS), mitral anomalies (mitral atresia, mitral hypoplasia), aortic anomalies (aortic arch hypoplasia, aortic coarctation, interrupted arch), Shone syndrome]; right heart malformations [Hypoplastic Right Heart Syndrome (HRHS), tricuspid valve alterations (tricuspid atresia), pulmonary anomalies (pulmonary atresia, pulmonary stenosis, pulmonary dysplasia)]; septal defects (complete atrioventricular canal defect, partial atrioventricular canal defect, interventricular septal defect); venous return anomalies (ductus venosus agenesis, inferior vena cava agenesis, persistent left superior vena cava); situs anomalies (left isomerism); complex anomalies (univentricular heart, double inlet or outlet ventricle); cardiac tumor (rhabdomyoma) and other cardiac anomalies [cardiomyopathies (dilated, hypertrophic, non-compaction cardiomyopathy) pericardial effusion, asymmetric cardiac chambers, dilated ascending aorta, hypoplastic aorta].

- Thoracic anomalies: Chest wall anomalies (hypoplastic thorax, ectopia *cordis*, fused ribs); Congenital diaphragmatic hernia (right-sided congenital diaphragmatic hernia, left-sided congenital diaphragmatic hernia); Hydrothorax; Pulmonary hypoplasia (pulmonary agenesis);

Thoracic mass (teratoma, congenital pulmonary airway malformation, pulmonary sequestration).

- Gastrointestinal anomalies: Intestinal anomalies (esophagus atresia, duodenal atresia, intestinal calcifications, bowel obstruction, bowel dilatation, rectal atresia, anal malformation, ascites, cyst); Hyperechogenic bowel; Abdominal wall defects (gastroschisis, omphalocele, teratoma, other abdominal wall defects); Hepatic anomalies (hepatic calcifications, hepatomegaly); Situs anomalies (situs inversus, isomerism); Pancreatic dysplasia.

- Facial anomalies: Mouth and lips anomalies (lip/palate unilateral cleft, bilateral cleft, and central cleft) Face and profile anomalies (micro retrognathia, dysmorphic face, other profile anomalies); Subcutaneous edema; Cervical anomalies (cystic hygroma, cervical lymphangiomas, nuchal edema, cervical tumor); nose anomalies (hypoplastic nose, nasal bone agenesis), ocular anomalies (hypertelorism, exophthalmos, eyelashes anomalies), external ear anomalies (implantation anomalies, hypoplastic external ear).

- Urologic anomalies: anomalies of renal position (pelvic kidney, horseshoe kidney), renal cystic diseases (isolated renal cyst, multicystic kidney disease, polycystic kidney disease), urinary tract dilation (UTD) (UTD A1, UTD A2-3),[201] duplicated collecting system, renal hyper echogenicity, renal malformation (renal agenesis, hypoplasia, and

dysplasia, complex genitourinary anomalies), bladder anomalies (bladder exstrophy, megacystis, others bladder anomalies).

- Genital anomalies: abnormal genitalia (ambiguous genitalia, other genital anomalies), penile anomalies (hypospadias, micropenis) and testicular anomalies (cryptorchidism).

- Skeletal: skeletal anomalies (limb body wall complex, micromyelia, focal femoral agenesis, reductional anomalies); digital anomalies (oligosyndactyly, polydactyly); spine anomalies (hemivertebra, other vertebral anomalies), skeletal dysplasia (hypochondroplasia, achondroplasia, osteochondrodysplasias, others skeletal dysplasias); foot anomalies (talipes equinovarus, minor foot anomalies).

- Hydrops: 2 or more fluid collections in the following districts: bowel (ascites), thorax (hydrothorax), heart (pericardial effusion), skin (subcutaneous edema)

5.8 STATISTICAL ANALYSIS

For the descriptive analysis, categorical variables were described as absolute frequency and percentage, while continuous data as a median and interquartile (IQR) range.

The chi-square test was used to compare the prevalence of pathogenic CNV and VUS among isolated cases, cases with associated abnormalities and associated FGR.

The software R was used for the statistical analysis. The significance level was set at 0.05.

PART 6

RESULTS

6 RESULTS

6.1 OVERALL

The total number of patients was 648 after 21 cases were excluded. Reasons of exclusions were: array failure (n=4, 19%); fetal infections: Citomegalovirus (n=4, 19%), Human Herpes Virus (n=1, 4.8%), Zika (n=1, 4.8%); common aneuploidies: Trisomy 13 (n=1, 4.8%), Trisomy 18 (n=3, 14.3%), Trisomy 21 (n=5, 23.8%), Trisomy 9 (n=1, 4.8%), Turner syndrome (n=1, 4.8%).

Demographic characteristics

The median maternal age was 33 years (IQR, 29 to 36), the median gestational age at invasive procedure was 21.3 weeks (IQR, 20 to 24.5). Three hundred and sixty-two were nulliparous (55.9%) and 286 (44.1%) were multiparous. Five hundred and ninety-three (91.5%) were singleton pregnancies, 36 (5.6%) were dichorionic twin pregnancies, 16 (2.5 %) were monochorionic twin pregnancies and 3 (0.5%) cases were triplet pregnancies.

Array CGH study

Considering the total cohort of anomalies included in our study, the overall prevalence of pathogenic CNV was 8.3% (54 cases out of 648), while the prevalence of VUS was 4.3 % (28 cases out of 648). In the 87.3% of cases (566 cases out of the total) the array ended up in a normal result. Table 3 describes the types of anomalies by system and the percentage of pathogenic CNV and VUS in both isolated and associated cases.

Table 3 Types of Anomalies and prevalence of pathogenic CNV and VUS

Anomaly	Total			Isolated			Associated		
	N	Pathogenic	VUS	N	Pathogenic	VUS	N	Pathogenic	VUS
CNS*	238	7.0%	8.0%	182	3.8%	7.7%	49	18.4%	8.2%
Cardiac	191	13.1%	3.7%	125	10.4%	1.6%	66	18.2%	7.6%
Thoracic	68	10.3%	5.9%	38	7.9%	5.3%	30	13.3%	6.7%
Gastrointestinal	89	5.6%	5.6%	47	2.1%	4.3%	42	9.5%	7.1%
Facial	66	16.7%	6.1%	22	0.0%	0.0%	44	25.0%	9.1%
Urinary	68	8.8%	1.5%	24	4.2%	4.2%	44	11.4%	0.0%
Genital	27	14.8%	3.7%	2	0.0%	0.0%	25	16.0%	4.0%
Skeletal	48	14.6%	6.3%	37	10.8%	2.7%	11	27.3%	18.2%
Hydrops	23	8.7%	8.7%	12	8.3%	0.0%	11	9.1%	18.2%

*CNS = Central Nervous System

6.2 STUDY 1: Central Nervous System

Two hundred and forty-four cases with CNS anomalies and array-CGH study were identified. From these, six cases were excluded, three had an abnormal QF-PCR (one case each of trisomy 21, 18 and 13), and three were diagnosed with a fetal infection (1 case of cytomegalovirus and 2 cases of Zika virus). Two hundred and thirty-eight cases were therefore included in the analysis (Figure 6).

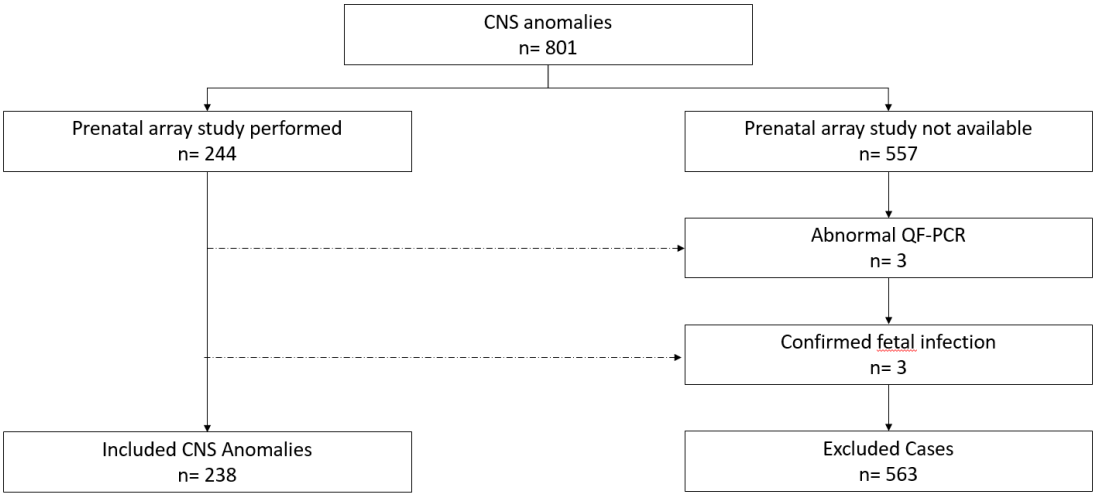


Figure 6: Central Nervous System (CNS) study. Flowchart: selection criteria

Demographic characteristics

The median maternal age was 33 years (IQR, 29 to 36), median gestational age at invasive testing was 21.5 weeks (IQR, 20 to 25). One hundred and twenty-eight women (53.8%) were nulliparous, and 110 (46.2%) were multiparous. Two hundred twenty-two (93.3%) were singleton pregnancies, 12 (5%) were dichorionic twin pregnancies, and 4 (1.7%) were monochorionic twin pregnancies.

Type of anomaly

Anomalies detected included ventriculomegaly (n=83, 34.9%); neural tube defects (n=62, 26.1%); midline anomalies (n=42, 17.6%); posterior fossa anomalies (n=31, 13.0 %); cortical development anomalies (n=13, 5.5%); hypoxic-ischemic or hemorrhagic lesions (n=2, 0.8%), intracranial cysts (n=3, 1.3%); brain tumor (n=1, 0.4%) and periventricular hyper echogenicity (n=1, 0.4%). Additional major non-CNS anomalies were detected in 49 cases (20.6%), including congenital heart defects (n=25, 10.5%);

facial dysmorphisms (n=10, 4.2%); thoracic anomalies (n=8, 3.4%); gastrointestinal or abdominal wall anomalies (n=7, 2.9%); renal anomalies (n=7, 2.9%); skeletal anomalies (n=10, 4.2%); fetal hydrops (n=2, 0.8%) and abnormal genitalia (n=8, 3.4%). Minor ultrasound anomalies were found in 31 cases (13%).

Fetal growth restriction was diagnosed in 16 cases (6.7%), of which 9 also had major abnormalities. In 182 cases (76.5%), an isolated CNS anomaly was detected.

Array-CGH study

In 225 (94.5%) cases, an amniocentesis was performed, in 10 (4.2%) a chorionic villous sampling and in 3 cases (1.3%), fetal tissue for chromosomal analysis was obtained following termination of pregnancy.

A pathogenic CNV was diagnosed in 16 cases (6.7%), VUS in 18 cases (7.6%), including 2 cases of probably pathogenic (CNVs that meet some but not all criteria to be considered pathogenic), and a normal result in 204 (85.7%) cases. Table 4 show pathogenic CNV and VUS according to the type, and the subgroup of CNS anomaly detected, either isolated or associated with other anomalies, respectively. A pathogenic CNV was found in 7 of the 182 (3.8%) cases of isolated anomalies, in 9 of the 49 (18.4%) that presented another major anomaly, and in none of the 7 cases with associated FGR (p=0.001). A VUS was found in 14 of the 182 (7.7%) with an isolated anomaly, in 4 (8.2%) of the 49 with associated major anomalies and in none of the 7 cases with FGR (p=0.741).

Considering the isolated cases with pathogenic CNVs or VUS that opted for a termination of pregnancy (in the case of VUS because of ultrasound findings), of the 14 prenatally isolated cases, in the post-mortem examinations were found: 1 case of additional

cerebral anomaly (case number 33 presented inferior vermis hypoplasia), and 1 case of extracerebral anomaly (case number 29 presented a double vagina). For those cases that opted for TOP with a normal array in which a post-mortem exam is available: of the 53 prenatally isolated cases without CNVs, in 7 cases, additional cerebral o extracerebral anomalies were found in the post-mortem examination (see

Table 5 and Table 6 for details). Table 7 and Table 8 report the description of each case with pathogenic CNV and VUS.

Table 4: Prevalence of pathogenic copy number variants (CNVs) and variants of unknown significance (VUS) in the different types of central nervous system (CNS) anomalies.

	ALL(n=238)			ISOLATED(n=182)			COMPLEX ABNORMALITIES(n=49)		
	N	PCNVs	VUS	N	PCNVs	VUS	N	PCNVs	VUS
Ventriculomegaly	83/238(34.9%)	7/83(8.4%)	6/83(7.2%)	63/182(34.7%)	3/63(4.8%)	4/63(6.3%)	16/49(32.7%)	4/16(25.0%)	2/16(12.5%)
Borderline/Mild	57/83(68.7%)	3/57(5.3%)	3/57(5.3%)	46/63(73%)	2/46(4.3%)	2/46(4.3%)	8/16(50%)	1/8(12.5%)	1/8(12.5%)
Moderate	12/83(14.5%)	3/12(25%)	1/12(8.3%)	9/63(14.3%)	1/9(11.1%)	1/9(11.1%)	2/16(12.5%)	2/2(100%)	-
Severe	14/83(16.9%)	1/14(7.1%)	2/14(14.3%)	8/63(12.7%)	0(0%)	1(12.5%)	6/16(37.5%)	1/6(16.7%)	1/6(16.7%)
Neural tube defects	62/238(26.1%)	2/62(3.2%)	3/62(4.8%)	58(31.89%)	2/58(3.4%)	2/58(3.4%)	4/49(8.2%)	0/4(0%)	1/4(25%)
Acrania	2/62(3.2%)	0(0%)	1(50%)	1/58(1.7%)	0(0%)	0(0%)	1/4(25%)	01/4	(25%)
Encephalocele	3/62(3.2%)	0(0%)	1(33.3%)	3/58(5.2%)	0(0%)	1(33.3%)	-	-	-
Spina bifida	57/62(93.5%)	2/57(3.5%)	1/57(1.8%)	54/58(93%)	2/54(3.7%)	1/54(1.9%)	3/4(75%)	0(0%)	0(0%)
Midline anomalies	42/238(17.6%)	2/42(4.8%)	3/42(7.1%)	32(17.6%)	0/32(0%)	3/32(9.4%)	10/49(20.4%)	2/10(20%)	0/10(0%)
Complete agenesis CC	22/42(52.4%)	0/22(0%)	1/22(4.5%)	18/32(56.3%)	0(0%)	1/18(5.6%)	4/10(40%)	0(0%)	0(0%)
Partial agenesis/dysgenesis of CC	10/42(23.8%)	0/10(0%)	1/10(10%)	8/32(25%)	0/8(0%)	1/8(12.5%)	2/10(20%)	0(0%)	0(0%)
Holoprosencephaly	6/42(14.3%)	2/6(33.3%)	1/6(16.7%)	3/32(9.4%)	0(0%)	1/3(33.3%)	3/10(30%)	2/3(66.7%)	0
CSP agenesis	4/42(9.5%)	0(0%)	0(0%)	3/32(9.4%)	0(0%)	0(0%)	1/10(10%)	0	0
Posterior fossa anomalies	31/238(13.0%)	4/31(12.9%)	4/31(12.9%)	17(9.3%)	2/17(11.8%)	3/17(17.6%)	13/49(26.5%)	2/13(15.4%)	1/13(7.7%)
Dandy Walker malformation	5/31(16.1%)	1/5(20%)	1/5(20%)	3/17(17.6%)	0(0%)	1(33.3%)	2/13(15.4%)	1/2(50%)	0

	ALL(n=238)			ISOLATED(n=182)			COMPLEX ABNORMALITIES(n=49)		
	N	PCNVs	VUS	N	PCNVs	VUS	N	PCNVs	VUS
Cerebellar hypoplasia	6/31(19.4%)	1/6(16.7%)	0(0%)	3/17(17.6%)	1/3(33.3%)	0(0%)	3/13(23.1%)	0	0
Vermian hypoplasia	2/31(6.5%)	0(0%)	0(0%)	0(0%)	-	-	2/13(15.4%)	0	0
Mega cisterna magna	8/31(25.8%)	2/8(25%)	1/8(12.5%)	5/17(29.4%)	1/5(20%)	0(0%)	2/13(15.4%)	1/2(50%)	1
Blake's pouch cyst	6/31(19.4%)	0(0%)	0/6(0%)	3/17(17.6%)	0(0%)	0/3(0%)	3/13(23.1%)	00	
Posterior fossa cyst	1/31(3.2%)	0(0%)	0(0%)	1/17(5.9%)	0(0%)	0(0%)	-	-	-
Rhombencephalosynapsis	3/31(9.7%)	0(0%)	2/3(66.7%)	2/17(11.8%)	0(0%)	2/2(100%)	1/13(7.7%)	0	0
Cortical development anomalies	13/238(5.5%)	1/13(7.7%)	2/13(15.4%)	6/182(3.3%)	0(0%)	2/6(33.3%)	5/49(10.2%)	1/5(20%)	0/5(0%)
Macrocephaly	2/13(15.4%)	0(0%)	0(0%)	1/6(16.7%)	0(0%)	0(0%)	1/5(20%)	0	0
Polymycrogyria	2/13(21.4%)	0(0%)	1/2(50%)	1/6(16.7%)	0(0%)	1/1(100%)	1/5(20%)	0	0
Abnormal sulcation/gyration	4/13(30.8%)	1/4(25%)	1/4(25%)	2/6(33%)	0(0%)	1/2(50%)	1/5(20%)	1/1(100%)	0
Microcephaly	5/13(38.5%)	0(0%)	0(0%)	2/6(33.3%)	0(0%)	0(0%)	2/5(40%)	0	0
hemorrhagic lesion	2/238(0.8%)	0/2(0%)	0/2(0%)	2(1.1%)	0(0%)	0(0%)	-	-	-
Brain hemorrhage	1/2(50%)	0(0%)	0(0%)	1/2(50%)	0(0%)	0(0%)	-	-	-
Venous sinus thrombosis	1/2(50%)	0(0%)	0(0%)	1/2(50%)	0(0%)	0(0%)	-	-	-
Intracranial cyst	3/238(1.3%)	0(0%)	0(0%)	3(1.6%)	0(0%)	0(0%)	-	-	-
Arachnoid cyst	3/3(100%)	0(0%)	0(0%)	3/3(100%)	0(0%)	0(0%)	-		-

	ALL(n=238)			ISOLATED(n=182)			COMPLEX ABNORMALITIES(n=49)		
	N	PCNVs	VUS	N	PCNVs	VUS	N	PCNVs	VUS
Periventricular hyperechogenicity	1(0.4%)	0(0%)	0(0%)	1(0.5%)	0(0%)	0(0%)	-		-
Tumor	1(0.4%)	0(0%)	0(0%)	0(0%)	-	-	1/49(2%)	0/1(0%)	0/1(0%)

Table 5. Postmortem exams with an abnormal prenatal array

Number of Case	US Prenatal diagnosis	Post-mortem examination cerebral findings	Post-mortem examination extra cerebral findings
32	Abnormal sulcation	Abnormal sulcation	None
18	Encephalocele	Encephalocele	None
33	Ventriculomegaly	Ventriculomegaly + inferior vermis hypoplasia	None
19	Hydrocephaly	Hydrocephaly	None
4	Cerebellar hypoplasia	Cerebellar hypoplasia	None
17	Alobar Holoprosencephaly	Alobar Holoprosencephaly	None
22	Abnormal sulcation + ventriculomegaly	Abnormal sulcation + ventriculomegaly	None

Number of Case	US Prenatal diagnosis	Post-mortem examination cerebral findings	Post-mortem examination extra cerebral findings
24	Agenesis corpus callosum + Polymicrogyria	Agenesis corpus callosum + Polymicrogyria	None
8	Ventriculomegaly	Autolysis of CNS	None
34	Partial agenesis of corpus callosum	Partial agenesis of corpus callosum	None
29	Neural tube defect (myelocele)	Neural tube defect (myelocele)	Double vagina
26	Dandy Walker (vermian agenesis)	Dandy Walker (vermian agenesis)	None
31	Rhombencephalosynapsis	Rhombencephalosynapsis	None
16	Neural tube defect (spina bifida)	Neural tube defect (spina bifida)	None

Table 6 Postmortem exams with a normal prenatal array

Number of Case	US Prenatal diagnosis	Post-mortem examination cerebral findings	Post-mortem examination of extracerebral findings
N1	Dandy-Walker	Dandy Walker + Partial ACC	
N2	Neural tube defect	Neural tube defect	Single umbilical artery
N3	Corpus callosum dysgenesis	Corpus callosum dysgenesis + abnormal sulcation	
N4	Neural tube defect	Neural tube defect	Retrognathia, anteverted nares
N5	Complete ACC	Complete ACC	Esophageal atresia with fistula type III. Aortic arch interruption type B, right-descending Aorta.
N6	Severe ventriculomegaly	Severe ventriculomegaly	Hemorrhagic lesions (adrenal glands, kidneys, liver, thymus)
N7	Encephalocele	Encephalocele	Renomegaly

ACC= agenesis of the corpus callosum

Table 7. Details of all cases with pathogenic CNVs. [202]

Case	Gestational age (weeks)	cardiac anomaly	Other anomalies	ISCN formula	Genetic content	Comments	De novo inherited	Test	visible by karyotype	Follow-up
1	BAC based array with low resolution	22	Ventriculomegaly	Pericardial effusion and right heart hypertrophy	6q26q27(163240985_170861575)x1 (7.54 Mb) 8p23.2p23.1(145466_7169549)x3 (7.02 Mb) 8p23.1(11269493_11998276)x3 (723 Kb)	Chromosome recurrent anomaly inv dup del(8p): ventriculomegaly. [203] CNS function and development genes DLGAP2 (OMIM 605438), CLN8 (OMIM 607837), ARHGEF10 (OMIM 608136). Congenital heart defects genes GATA4 (OMIM 600576) [203]	De novo	qChip Pre v1.1 Targeted	YES	TOP
2		16	Ventriculomegaly	Congenital diaphragmatic hernia, renal cyst	Xq26.2(132315039_13287 6911)x0 (0.56 Mb)	Ventriculomegaly, congenital diaphragmatic hernia, renal cyst: Simpson-Golabi-Behmel Syndrome Type 1. Genes, GPC4 (OMIM: 300168), GPC3 (OMIM: 300037)	Maternal	Agilent G4827A (CGH ISCA v2,8x60K)	NO	TOP
3		18	Dandy Walker malformation	Hypoplastic left heart with double outlet right ventricle; Cystic higroma	1q21.1q21.2(145415190-147380935)x3 1.96 Mb)	Complete 1q21.1 duplication (proximal + distal). q21 distal (BP3-BP4) duplication show variable penetrance of 17-47% and its known to be associated to brain and heary anomalies [204]	Paternal	Agilent G4827A (CGH ISCA v2,8x60K)	NO	TOP
4		20	Cerebellar hypoplasia	-	6q27(169099035_170911240)x1 (1.91 Mb)	Region associated to brain malformations [205] especially periventricular nodular heterotopia (PNH) [206]	Paternal balanced translocation (46,XY,t(6;13)(q27;p11.2)	qChip Pre v1.1 Complete	NO	TOP
5		21	Holoprosencephaly	Oligosyndactlyly; Fetal growth restriction	13q31.3q34(91571035_115093115)x1 (23.5Mb)	ZIC2 (OMIM 603073) 8.2% of holoprosencephaly (HPE) probands show ZIC2 (MIM603073) defects, mostly “de novo” (70% of cases) [207]. The overall penetrance of phenotypic manifestations (including microform HPE) due to mutations in ZIC2 is estimated to be 96% and the prevalence of brain anomalies is estimated to be 90% [208] . ZIC2 mutations are generally characterised by a normal face or a moderate facial dysmorphia associated with alobar or semilobar HPE [207].	De novo	ogt 020045 (CytoSure Constitutional v3 array8x60K)	YES	TOP
6		20	Abnormal sulcation	Cleft lip	1p36.33p36.32(794596_4458182)x1 (3.57 Mb)	Brain anomalies and orofacial clefting are part of the 1p36 deletion syndrome [209].	De novo	qChip Pre v1.1 Targeted	NO	TOP
7		20	Megacisterna magna	Nuchal edema	3q29(193892289_196215670)x3 (2.3 Mb)	Enlarged cisterna magna has been described in 3q29 syndrome.[38]	Maternal	qChip Pre	NO	Severe developmental delay and epilepsy (4 years)

Case	Gestational age (weeks)	cardiac anomaly	Other anomalies	ISCN formula	Genetic content	Comments	De novo inherited	Test	visible by karyotype	Follow-up
8		25	Ventriculomegaly	-	5q21.3q23.1 (105029588_120102372)x1 (15 Mb)	5q deletion includng APC (OMIM:611731) gene is associated to risk of adenomatous polyposis, (MIM175100) and a variable and a phenotypically poor defined phenotipic syndrome including dysmorphic features, and mild mental retardation [39].	De novo	qChip Pre v1.1 Complete	YES	TOP
9		17	Megacisterna magna	Pulmonary stenosis, hydrothorax, ascites; hyperechogenic kidneys; micrognathia	9p24.3p13.1(204090_38704041)x1 (38.5 Mb)	DMRT1 DOCK8 Small telomeric 9p24.3 deletions including DMRT genes (DMRT1 bring the strongest candidate gene for sex reversal) cause genital anomalies in male subjects, ranging from disorder of gonadal sex to genital differentiation anomalies. More proximal, interstitial 9p22.3-p24.1 deletions result in a malformation syndrome characterized by intellectual disability, congenital hypotonia and a range of cranio-facial abnormalities, less frequently cardiac defects, epilepsy, inguinal hernia, omphalocele, choanal atresia, scoliosis and non-ketotic hypoglycaemia [40] .	De novo	- qChip Pre v1.1 Complete	YES	TOP
10		25	Spina bifida	-	15q25.2q25.3(84084071_86870834)x1 (2.8 Mb)	15q25.2 distal deletions should be considered as a susceptibility locus for variable neurodevelopmental disorders with high risk than proximal deletions for neuropsychiatric disorders, seizures, hypotonia, and strabism. One patient has dysmorphic features similar to Noonan syndrome [41] .	De novo	qChip Pre v1.1 Targeted	NO	Normal neurodevelopment
11		27	Ventriculomegaly	-	10q11.22q11.23(47596461_51798957)x1 (4.4 Mb)	10q11.2 deletions are associated to brain abnormlities: 19% patients described have microcephaly and 18%, corpus callosum abnormalities [42]	unknown origin	qChip Pre v1.1 Complete	NO	No follow up
12		32	Ventriculomegaly	Hidrops; Polihydramnios	17q12(34856055_36777884)x1 (1.9 Mb)	The 17q12 recurrent deletion syndrome is characterized by structural or functional abnormalities of the kidney and urinary tract (80-85% affected individuals) [43]	Declined study	qChip Pre v1.1 Complete	NO	Neonatal death
13		24	Ventriculomegaly	-	5q35.2 _ q35.3(175667946_177422740)x3 (1.75Mb)	The microduplication of this 5q35.2q35.3 region encompassing NSD1 gene, is associated with what has been described as a "reversed" Sotos syndrome phenotype, commonly including short stature, microcephaly, delayed bone age, and Developmental Delay/Intellectual Disability (DD/ID).	De novo	ogt 020045 (CytoSure Constitutional v3 array (8x60K)	NO	Live birth,no follow up

Case	Gestational age (weeks)	cardiac anomaly	Other anomalies	ISCN formula	Genetic content	Comments	De novo inherited	Test	visible by karyotype	Follow-up
14		25	Holoprosencephaly	Ventricular septum defect; Fetal growth restriction	13q31.3 _ q34(92587094_115093115)x1 (22.5 Mb)	Deletion includes ZIC2 (MIM603073). Defects in ZIC2 cause holoprosencephaly (HPE) [207].	De novo	ogt 020045 (CytoSure Constitutional v3 array 8x60K)	YES	TOP
15	Not mosaicism evidence in chorion villus array and karyotype analysis.	13	Hydrocephaly; abnormal posterior fossa	Cardiac defect (not specified); Exomphalos; Nuchal edema	(9)x3	Mosaic trisomy 9 is a rare Nuchal abnormalities, central nervous system, and cardiac are common findings in trisomy of chromosome 9 [44210]	Not studied	ogt 020045 (CytoSure Constitutional v3 array8x60K)	YES	TOP
16		18	Neural tube defect (spina bifida)	-	Xq27.1(139131377_139724080)x2 (592Kb)	SOX3 (OMIM: 313430) duplication has been recently associated with neural tube defects [211,212].	Not studied	qChip Pre Complete	NO	TOP

Table 8. Details of all cases with VUS [202]

Case	Gestational age (weeks)	cardiac anomaly	Other anomalies	ISCN formula	Genetic content	Comments	De novo inherited	Test	visible by karyotype	Follow-up
17		13.3	Alobar Holoprosencephaly	-	4p16.3(45889_127278)x3 (81 Kb)	ZNF595, ZNF718	Not studied	ogt 020045 (CytoSure Constitutional v3 array 8x60K	NO	TOP
18		14	encephalocele	Cystic higroma	10q11.22(46699438_49263598)x3 (2.56 Mb)	26 genes	Not studied	Agilent G4827A (CGH ISCA v2,8x60K	NO	TOP
19		20	Hidrocephaly		1q21.2(150619114_151174820)x3 (556Kb) Arr 7p22.2(2759448_2803743)x1 (44Kb)	CTKS,ARNT,MLLT1 GNA12	Paternal (both C**NVs)**	qChip post	NO	TOP
20	†	21	Ventriculomegaly	Tetralogy of fallot; microretrognathia; clubfoot	2q21.2(134067405_134295740)x1 (228Kb) 14q11.2(22409129_23167893)x3 (759Kb)	NAP5 DAD1,ABHD4	Not studied	qChip post		TOP
21		30	Ventriculomegaly	-	2p16.1(55281597_56633505)x3 (1.35 Mb)	13 genes	Maternal	Agilent G4827A (CGH ISCA v2, 8x60K	NO	Normal neurodevelopment 1 year FO
22	†	21	Abnormal sulcation	Ventriculomegaly	3q29(196263081_196825905)x3 (0.56 Mb) (VUS because includes only 50% of 3q29 duplication syndrome)	12 genes (PAK2)	Paternal (normal phenotype)	020045 (CytoSure Constitutional v3 array 8x60K	NO	TOP
23		30	Ventriculomegaly	Fetal growth restriction,	Xp22.31(7744144_8435991)x2 (540Kb)	NA	Not studied	qChip pre	NO	Ventriculomegaly; brainstem disgenesis; complex cardiopathy
24		Post TOP	Agenesis corpus callosum	Polymicrogyria	7q21.3(93039264_93234125)x1 (195Kb)	CALCR	Not studied	qChip post	NO	TOP
25		29	Ventriculomegaly	-	Xp22.13(18609619_19030114)x2 (420Kb)	RS1, PPEF1, PHKA2, RefSeq (CDKL5,GPR64)	Maternal	qChip Pre Complete	NO	Partial syndactyly foot ; Normal neurodevelopment FO 7 months
26		18	Dandy Walker	-	Xq21.31(87877030_88253336)x2 (376Kb)	CPXCR1	Maternal	qChip post	NO	TOP

Case	Gestational age (weeks)	cardiac anomaly	Other anomalies	ISCN formula	Genetic content	Comments	De novo inherited	Test	visible by karyotype	Follow-up
			(vermian agenesis)							
27		28	Megacisterna magna	Tricuspid valve dysplasia	11q14.1(81827270_83453945)x3 (1.6Mb) 17p11.2(19572563_19790651)x3 (218Kb)	DLG2, PCRP, RAB30,ANKRD42 ALDH3A2,SLC47A2,ALDH3A1,ULK2	Maternal	qChip Pre v1.1 Targeted	NO	Neuronal heterotopias
28		20	Anencephaly	Ventricular septum defect; single umbilical artery	16p13.11(15551302_16194578)x3 (643 Kb)	C16orf45, KIAA0430, NDE1, MIR484, MYH11, C16orf63, ABCC1	Not studied	Agilent G4827A (CGH ISCA v2, 8x60K	NO	TOP
29		21	Neural tube defect (myelocele)	-	Xp22.31(7810940_8434361)x2 (623 Kb)	VCX, PNPLA4, MIR651, VCX2, VCX3B	Not studied	ogt 020045 (CytoSure Constitutional v3 array8x60K	NO	TOP
30		25	rhombencephalosynapsis	-	Xq21.1(77082384_77139044)x3 (57 Kb)	MAGT1	Not studied	ogt 020045 (CytoSure Constitutional v3 array 8x60K	NO	Rhombencephalosynapsis (suspect Gomez-Lopez-Hernandez Snd)
31		21	rhombencephalosynapsis	-	11q24.3(128170803_128554700)x3 (384 Kb)	ETS1	Not studied	ogt 020045 (CytoSure Constitutional v3 array 8x60K	NO	TOP
32		27	Abnormal sulcation	-	17p13.3(810138_912611)x1 (102 Kb)	NXN, TIMM22, ABR	Not studied	ogt 020045 (CytoSure Constitutional v3 array 8x60K	NO	TOP
33		22	Ventriculomegaly	-	12q14.3(65564724_65671221)x3 (106 Kb)	LEMD3	Not studied	ogt 020045 (CytoSure Constitutional v3 array 8x60K	NO	TOP
34		22	Partial agenesis of corpus callosum	-	13q12.11q12.12(20100790_25458788)x1 (5.3 Mb)	>30	De novo	qChip Pre v1.1 Targeted	doubtful	TOP

6.3 STUDY 2: CARDIAC ANOMALIES

One hundred nighty-nine cases with cardiac anomalies and array-CGH study were identified. From these, 8 cases were excluded for the final analysis: five presented an abnormal QF-PCR (3 cases of trisomy 21, and one case each of trisomy 18 and 13), two cases were diagnosed with a fetal infection (1 case of cytomegalovirus and 1 case of Zika virus), and in one case a failure in the array analysis occurred. One hundred and nighty-one cases were therefore included in the analysis.

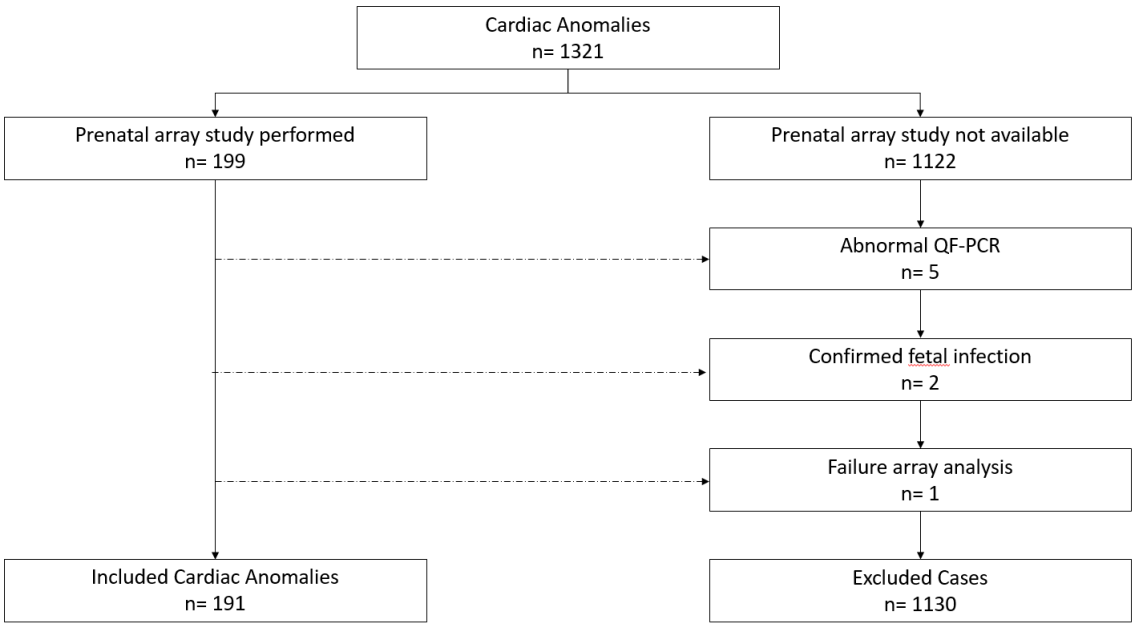


Figure 7: Cardiac anomalies study. Flowchart: selection criteria

Demographic characteristics

For the cardiac anomalies group, the median maternal age was 32 years (IQR, 16 to 44). At invasive test, the median gestational age was 21.6 weeks (IQR, 11.2 to 36.1); one hundred and ten (57.6 %) women were nulliparous, the remaining 81 (42.4%) women

were multiparous. Referring to the type of pregnancy, 175 (91.6%) were singleton pregnancies, 8 (4.2%) were dichorionic twin pregnancies, and 8 (4.2%) were monochorionic twin pregnancies.

Type of anomaly

A cardiac anomaly was diagnosed as isolated in 125 (65.4%) out of the 191 cases; conversely, the cardiac abnormality was present with one more associated anomaly in 66 (34.6%) of the 191 cases.

A growth restriction was diagnosed in 20 cases (10.5%). All these cases were present in the subgroup with major associated anomalies; the remaining 171 (89.5%) cases did not present fetal growth alterations.

Cardiac anomalies identified were classified into groups as follows: conotruncal malformations (n=54, 28.3%), left heart malformations (n=29, 15.2%), right heart malformations (n=19, 9.9%), septal defects (n=20, 10.5%), venous return anomalies (n=38, 19.9%), situs anomalies (n=2, 1.0%), complex anomalies (n=12, 6.3%), cardiac tumor (n=1, 0.5%) and other cardiac anomalies (n=16, 8.4%).

When only isolated cardiac anomalies were considered (n=125, 65.4%) the groups of abnormalities were represented as follows: cardiac tumor (n=1, 0.8%), complex anomalies (n=7, 5.6%), conotruncal malformations (n=41, 32.8%), left heart malformations (n=18, 14.4%), other cardiac anomalies (n=9, 7.2%), right heart disease (n=12, 9.6%), septal defects (n=8, 6.4%), situs anomalies (n=2, 1.6%), venous return anomalies (n=27, 21.6%).

In case of cardiac anomalies associated to extracardiac alterations (including the 20 cases of fetal growth restriction) the anomalies found were: complex anomalies (n=5, 7.5%), conotruncal malformations (n=13, 19.6%), left heart malformations (n=11, 16.6%), other cardiac anomalies (n=7, 10.6%), right heart malformations (n=7, 10.6%), septal defects (n=12, 18.1%), venous return anomalies (n=11, 16.6%); no cardiac tumors or situs anomalies were found associated to extra cardiac anomalies.

Array study

In 174 cases (91.1%), an amniocentesis was performed, in 14 (7.3%) cases a chorionic villous sampling, in 2 cases (1.0 %), a fetal tissue sample (biopsy) for chromosomal analysis was obtained following termination of pregnancy, and in one case (0.5%) fetal cell for the analysis were obtained from fetal blood. Considering the result of the array, a pathogenic CNV was diagnosed in 25 cases (13.1%), a VUS in 7 cases (3.7%), and a normal result was obtained in 159 (83.2 %) cases.

Table 9 describes all pathogenic CNVs and VUS findings for the different cardiac anomalies, both for isolated and those with extracardiac associated anomalies.

If isolated cardiac anomalies were considered, in 13 of the 125 (10.4%) cases presented a pathogenic CNV; conversely, in 12 of the 66 (18.2%) cases of cardiac anomaly with associated major anomaly and/or associated FGR, a pathogenic CNV was diagnosed.

A VUS was found in 2 of the 125 (1.6%) cases with an isolated anomaly, and in 5 (7.6%) of the 66 cases with associated major anomalies and FGR.

Table 10 reports the description of each case with pathogenic CNV.

Table 9. Prevalence of pathogenic copy number variants (CNVs) and variants of unknown significance (VUS) in the different types of cardiac anomalies.

	ALL(n=191)			ISOLATED(n=125)			ASSOCIATED(n=66)		
	N	Pathogenic	VUS	N	Pathogenic	VUS	N	Pathogenic	VUS
Cardiac tumor	1/191 (0.52%)	0/1 (0%)	0/1 (0%)	1/125 (0.8%)	0/1 (0%)	0/1 (0%)	0/66 (0%)	0/0 (0%)	0/0 (0%)
Rabdomyoma	1/1 (100%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)
Complex	12/191 (6.28%)	1/12 (8.33%)	1/12 (8.33%)	7/125 (5.6%)	0/7 (0%)	0/7 (0%)	5/66 (7.57%)	1/5 (20%)	1/5 (20%)
Univentricular heart	3/12 (25%)	0/3 (0%)	0/3 (0%)	3/7 (42.8%)	0/3 (0%)	0/3 (0%)	0/5 (0%)	0/0 (0%)	0/0 (0%)
Double inlet ventricle	1/12 (8.33%)	0/1 (0%)	0/1 (0%)	1/7 (14.2%)	0/1 (0%)	0/1 (0%)	0/5 (0%)	0/0 (0%)	0/0 (0%)
Double outlet ventricle	8/12 (66.6%)	1/8 (12.5%)	1/8 (12.5%)	3/7 (42.8%)	0/3 (0%)	0/3 (0%)	5/5 (100%)	1/5 (20%)	1/5 (20%)
Conotruncal disease	54/191 (28.2%)	7/54 (12.9%)	2/54 (3.70%)	41/125 (32.8%)	6/41 (14.6%)	0/41 (0%)	13/66 (19.6%)	1/13 (7.69%)	2/13 (15.3%)
Aortic override	5/54 (9.25%)	1/5 (20%)	0/5 (0%)	0/41 (0%)	0/0 (0%)	0/0 (0%)	5/13 (38.4%)	1/5 (20%)	0/5 (0%)
Right aortic arch	8/54 (14.8%)	1/8 (12.5%)	1/8 (12.5%)	7/41 (17.0%)	1/7 (14.2%)	0/7 (0%)	1/13 (7.69%)	0/1 (0%)	1/1 (100%)
Aberrant right subclavian artery (ARSA)	3/54 (5.55%)	0/3 (0%)	0/3 (0%)	1/41 (2.43%)	0/1 (0%)	0/1 (0%)	2/13 (15.3%)	0/2 (0%)	0/2 (0%)
Tetralogy of Fallot	17/54 (31.4%)	4/17 (23.5%)	1/17 (5.88%)	13/41 (31.7%)	4/13 (30.7%)	0/13 (0%)	4/13 (30.7%)	0/4 (0%)	1/4 (25%)
Great arteries transposition	19/54 (35.1%)	1/19 (5.26%)	0/19 (0%)	18/41 (43.9%)	1/18 (5.55%)	0/18 (0%)	1/13 (7.69%)	0/1 (0%)	0/1 (0%)
Truncus arteriosus	2/54 (3.70%)	0/2 (0%)	0/2 (0%)	2/41 (4.87%)	0/2 (0%)	0/2 (0%)	0/13 (0%)	0/0 (0%)	0/0 (0%)
Aorto-pulmonary window	1/54 (1.85%)	0/1 (0%)	0/1 (0%)	1/41 (2.43%)	0/1 (0%)	0/1 (0%)	0/13 (0%)	0/0 (0%)	0/0 (0%)
Left heart defects	29/191 (15.1%)	5/29 (17.2%)	1/29 (3.44%)	18/125 (14.4%)	3/18 (16.6%)	1/18 (5.55%)	11/66 (16.6%)	2/11 (18.1%)	0/11 (0%)

	ALL(n=191)			ISOLATED(n=125)			ASSOCIATED(n=66)		
	N	Pathogenic	VUS	N	Pathogenic	VUS	N	Pathogenic	VUS
Hypoplastic Left Heart Syndrome	13/29 (44.8%)	1/13 (7.69%)	1/13 (7.69%)	9/18 (50%)	1/9 (11.1%)	1/9 (11.1%)	4/11 (36.3%)	0/4 (0%)	0/4 (0%)
Aortic arch hypoplasia	5/29 (17.2%)	1/5 (20%)	0/5 (0%)	3/18 (16.6%)	0/3 (0%)	0/3 (0%)	2/11 (18.1%)	1/2 (50%)	0/2 (0%)
Interrupted arch	2/29 (6.89%)	1/2 (50%)	0/2 (0%)	1/18 (5.55%)	1/1 (100%)	0/1 (0%)	1/11 (9.09%)	0/1 (0%)	0/1 (0%)
Aortic coarctation	6/29 (20.6%)	0/6 (0%)	0/6 (0%)	4/18 (22.2%)	0/4 (0%)	0/4 (0%)	2/11 (18.1%)	0/2 (0%)	0/2 (0%)
Shone syndrome	1/29 (3.44%)	1/1 (100%)	0/1 (0%)	1/18 (5.55%)	1/1 (100%)	0/1 (0%)	0/11 (0%)	0/0 (0%)	0/0 (0%)
Mitral atresia	1/29 (3.44%)	0/1 (0%)	0/1 (0%)	0/18 (0%)	0/0 (0%)	0/0 (0%)	1/11 (9.09%)	0/1 (0%)	0/1 (0%)
Mitral hypoplasia	1/29 (3.44%)	1/1 (100%)	0/1 (0%)	0/18 (0%)	0/0 (0%)	0/0 (0%)	1/11 (9.09%)	1/1 (100%)	0/1 (0%)
Other	16/191 (8.37%)	1/16 (6.25%)	2/16 (12.5%)	9/125 (7.2%)	0/9 (0%)	1/9 (11.1%)	7/66 (10.6%)	1/7 (14.2%)	1/7 (14.2%)
Asymmetric cardiac chambers	3/16 (18.7%)	0/3 (0%)	0/3 (0%)	1/9 (11.1%)	0/1 (0%)	0/1 (0%)	3/7 (42.8%)	0/3 (0%)	0/3 (0%)
Dilated ascending aorta	1/16 (6.25%)	0/1 (0%)	0/1 (0%)	1/9 (11.1%)	0/1 (0%)	0/1 (0%)	0/7 (0%)	0/0 (0%)	0/0 (0%)
Hypoplastic aorta	2/16 (12.5%)	0/2 (0%)	0/2 (0%)	2/9 (22.2%)	0/2 (0%)	0/2 (0%)	0/7 (0%)	0/0 (0%)	0/0 (0%)
Dilated cardiomyopathy	3/16 (18.7%)	1/3 (33.3%)	1/3 (33.3%)	2/9 (22.2%)	0/2 (0%)	1/2 (50%)	1/7 (14.2%)	1/1 (100%)	0/1 (0%)
Hypertrophic cardiomyopathy	2/16 (12.5%)	0/2 (0%)	0/2 (0%)	0/9 (0%)	0/0 (0%)	0/0 (0%)	2/7 (28.5%)	0/2 (0%)	0/2 (0%)
Non-compaction cardiomyopathy	3/16 (18.7%)	0/3 (0%)	0/3 (0%)	2/9 (22.2%)	0/2 (0%)	0/2 (0%)	2/7 (28.5%)	1/2 (50%)	0/2 (0%)
Pericardial effusion	2/16 (12.5%)	0/2 (0%)	1/2 (50%)	1/9 (11.1%)	0/1 (0%)	0/1 (0%)	1/7 (14.2%)	0/1 (0%)	1/1 (100%)
Right heart defects	19/191 (9.94%)	4/19 (21.0%)	1/19 (5.26%)	12/125 (9.6%)	2/12 (16.6%)	0/12 (0%)	7/66 (10.6%)	2/7 (28.5%)	1/7 (14.2%)

	ALL(n=191)			ISOLATED(n=125)			ASSOCIATED(n=66)		
	N	Pathogenic	VUS	N	Pathogenic	VUS	N	Pathogenic	VUS
Hypoplastic Right Heart Syndrome	5/19 (26.3%)	1/5 (20%)	0/5 (0%)	3/12 (25%)	0/3 (0%)	0/3 (0%)	2/7 (28.5%)	1/2 (50%)	0/2 (0%)
Tricuspid dysplasia	1/19 (5.26%)	0/1 (0%)	1/1 (100%)	0/12 (0%)	0/0 (0%)	0/0 (0%)	1/7 (14.2%)	0/1 (0%)	1/1 (100%)
Pulmonary atresia	4/19 (21.0%)	2/4 (50%)	0/4 (0%)	3/12 (25%)	2/3 (66.6%)	0/3 (0%)	1/7 (14.2%)	0/1 (0%)	0/1 (0%)
Pulmonary dysplasia	2/19 (10.5%)	0/2 (0%)	0/2 (0%)	1/12 (8.33%)	0/1 (0%)	0/1 (0%)	1/7 (14.2%)	0/1 (0%)	0/1 (0%)
Pulmonary stenosis	5/19 (26.3%)	1/5 (20%)	0/5 (0%)	4/12 (33.3%)	0/4 (0%)	0/4 (0%)	1/7 (14.2%)	1/1 (100%)	0/1 (0%)
Tricuspid atresia	2/19 (10.5%)	0/2 (0%)	0/2 (0%)	1/12 (8.33%)	0/1 (0%)	0/1 (0%)	1/7 (14.2%)	0/1 (0%)	0/1 (0%)
Septal defects	20/191 (10.4%)	5/20 (25%)	0/20 (0%)	8/125 (6.4%)	1/8 (12.5%)	0/8 (0%)	12/66 (18.1%)	4/12 (33.3%)	0/12 (0%)
Complete atrioventricular canal defect	3/20 (15%)	0/3 (0%)	0/3 (0%)	1/8 (12.5%)	0/1 (0%)	0/1 (0%)	2/12 (16.6%)	0/2 (0%)	0/2 (0%)
Partial atrioventricular canal defect	1/20 (5%)	0/1 (0%)	0/1 (0%)	1/8 (12.5%)	0/1 (0%)	0/1 (0%)	0/12 (0%)	0/0 (0%)	0/0 (0%)
Ventricular septal defects	16/20 (80%)	5/16 (31.2%)	0/16 (0%)	6/8 (75%)	1/6 (16.6%)	0/6 (0%)	10/12 (83.3%)	4/10 (40%)	0/10 (0%)
Situs anomalies	2/191 (1.04%)	0/2 (0%)	0/2 (0%)	2/125 (1.6%)	0/2 (0%)	0/2 (0%)	0/66 (0%)	0/0 (0%)	0/0 (0%)
Left isomerism	2/2 (100%)	0/2 (0%)	0/2 (0%)	2/2 (100%)	0/2 (0%)	0/2 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)
Venous return anomalies	38/191 (19.8%)	2/38 (5.26%)	0/38 (0%)	27/125 (21.6%)	1/27 (3.70%)	0/27 (0%)	11/66 (16.6%)	1/11 (9.09%)	0/11 (0%)
Ductus venosus agenesis	15/38 (39.4%)	1/15 (6.66%)	0/15 (0%)	11/27 (40.7%)	0/11 (0%)	0/11 (0%)	4/11 (36.3%)	1/4 (25%)	0/4 (0%)
Inferior vena cava agenesis	2/38 (5.26%)	0/2 (0%)	0/2 (0%)	0/27 (0%)	0/0 (0%)	0/0 (0%)	2/11 (18.1%)	0/2 (0%)	0/2 (0%)
PVCSI	21/38 (55.2%)	1/21 (4.76%)	0/21 (0%)	16/27 (59.2%)	1/16 (6.25%)	0/16 (0%)	5/11 (45.4%)	0/5 (0%)	0/5 (0%)

Table 10. Details of all cases with pathogenic CNVs in the different types of cardiac anomalies.

Case	Gestational age (weeks)	cardiac anomaly	Other anomalies	ISCN formula	Genetic content	Comments	De novo inherited	Test	visible by karyotype	Follow-up
1	22	dilated cardiomyopathy	cerebral ventriculomegaly	6q26q27(163240985_170861575)x1 , 8p23.2p23.1(145466_7169549)x3, 8p23.1(11269493_11998276)x3	Chromosome anomaly inv dup del(8p)	The three CNVs are part of the same chromosome recurrent anomaly inv dup del(8p): ventriculomegaly. [203] CNS function and development genes DLGAP2 (OMIM 605438), CLN8 (OMIM 607837), ARHGEF10 (OMIM 608136). Congenital heart defects genes GATA4 (OMIM 600576)	de novo	Low resolution BAC array	YES	TOP
2	21.4	right aortic arch	isolated	22q11.21(18894820_21457610)x1	Proximal 22q11.2 deletion (classic, LCR22A-LCR22D) (DiGeorge/velocardiofacial syndromes)	22q11.2 deletion syndrome	de novo	High resolution 8x60 oligonucleotide ISCA array	NO	TOP
3	18	hyperteloris m	Dandy Walker malformation, Hypoplastic left heart with double outlet right ventricle; Cystic higroma	1q21.1q21.2(145415190_147380935)x3	1q21.1 proximal duplication TAR region (RBM8A) + 1q21.1 distal duplication (BP3-BP4, GJA5)	Complete 1q21.1 duplication (proximal + distal). q21 distal (BP3-BP4) duplication shows variable penetrance of 17-47% and its known to be associated to brain and heary anomalies	paternal	High resolution 8x60 oligonucleotide ISCA array)	NO	TOP
4	21	tetralogy of Fallot	isolated	22q11.21(18894820_21457610)x1	Proximal 22q11.2 deletion (classic, LCR22A-LCR22D) (DiGeorge/velocardiofacial syndromes)	22q11.2 deletion syndrome	de novo	High resolution 8x60 oligonucleotide ISCA array	NO	TOP
5	22	great arteries transposition	isolated	17q12(34856055_362489178)x3	17q12 duplication syndrome (HNF1B)	17q12 duplication syndrome (HNF1B)	maternal	High resolution 8x60 oligonucleotide ISCA array	NO	Newborn with great arteries transposition and criptorquidism
6	22.4	tetralogy of Fallot	isolated	2q13(111646676_113065741)x1	2q13 deletion syndrome	Deletion has an incomplete penetrance, since healthy carriers have been described [213] and patients with developmental retardation / intellectual disability autism spectrum disorder ,speech delay,hypotonia,congenital heart defects,dysmorphic features [214]. Combination of a deleterious variant in TMEM87B with an hemizygous 2q13 microdeletion suggests a recessive condition characterized by congenital heart disease and restrictive cardiomyopathy. The penetrance is variable, since healthy carriers have been described	maternal	High resolution 8x60 oligonucleotide ISCA array	NO	TOP
7	13.1	Hypoplastic Left Heart Syndrome	cystic higroma	4p16.3p15.1(75647_35238188)x1, 4p15.2p15.1(	4p16.3 deletion (Wolf-Hirschhorn syndrome)		de novo	High resolution 8x60 oligonucleotide ISCA array	YES	TOP

Case	Gestational age (weeks)	cardiac anomaly	Other anomalies	ISCN formula	Genetic content	Comments	De novo inherited	Test	visible by karyotype	Follow-up
				25428634_33 08093)x1						
8	21	PLSVC	isolated	15q24.1q24.2 (74562813_ 75954617)	Witteveen-Kolk syndrome (MIM: 613406) gene SIN3 (MIM:607776)	The 15q24 chromosome region is flanked by five segmental duplication blocks (SD) from centromere to telomere named LCR15q24A (BP4), LCR15q24B (BP1), LCR15q24C, LCR15q24D (BP2) and LCR15q24E (BP3) which have been implicated in the 15q24 chromosome rearrangements via non allelic homologous recombinations. The 15q24 microdeletion have very variable breakpoints, frequently inside lowcopy repeats (LRs) 15q24A-E. Most individuals with 15q24 microdeletion had a 3.1 Mb deletion located between LCR15q24A and LCR15q24D while the remaining cases frequently carriy the smaller deletion of approximately 2.6 Mb extending from LCR15q24A to LCR15q24C. Moreover, rare atypical 15q24 losses with only one or no breakpoints within segmental duplications have also been recorded [215,216].	de novo	High resolution 8x60 oligonucleotide ISCA array	NO	TOP
9	21	tetralogy of Fallot	isolated	17p12p11.2(15162481_20524013)x3, 22q11.21(18894835_21809009)x1	17p11.2 duplication (Potocki-Lupski syndrome); Proximal 22q11.2 deletion (classic, LCR22A-LCR22D), frequent (90%)(DiGeorge/velocardiofacial syndromes)	2 syndromes	de novo	High resolution 8x60 oligonucleotide ISCA array	NO	TOP
10	17.3	pulmonary stenosis	Megacisterna magna,hydrothorax, ascites;	9p24.3p13.1(204090_38704041)x1	9p24.3p13.1 terminal deletion	DMRT1 DOCK8 , interstitial 9p22.3-p24.1 deletions result in a malformation syndrome characterized by intellectual disability, congenital hypotonia and a range of cranio-facial abnormalities, less frequently cardiac defects, epilepsy, inguinal hernia, omphalocele, choanal atresia, scoliosis and non-ketotic hypoglycaemia [217]	de novo	qChip Pre v1.1 Complete	YES	TOP
11	32	aortic override	isolated	10p12.31p11.23(20588978_30379604)x1, 10p11.23p11.22(30379604_32752603)x3	10p12p11 syndrome (developmental delay, abnormal behaviour, dysmorphic features, visual impairments and cardiac malformations.		de novo	High resolution 8x60 oligonucleotide ISCA array	NO	TOP
12	17.2	tetralogy of Fallot	isolated	20q13.13q13.33(49349453_62904501)x3	20q13.2 duplication		de novo		YES	TOP
13	24	ductus venosus agenesis	abnormal ear implantation	3p25.2 x1	RAF1 gene			High resolution 8x60	NO	Neonatal death

Case	Gestational age (weeks)	cardiac anomaly	Other anomalies	ISCN formula	Genetic content	Comments	De novo inherited	Test	visible by karyotype	Follow-up
								oligonucleotide ISCA array		
14	26	double outlet ventricle	renal displasia	(14)x2~3	mosaic trisomy chromosome 2			High resolution 8x60 oligonucleotide ISCA array	YES	TOP
15	17	pulmonary atresia		22q11.21(18765102_21457610)x1	Proximal 22q11.2 deletion (classic, LCR22A-LCR22D) (DiGeorge/velocardiofacial syndromes)	22q11.2 deletion syndrome	de novo	High resolution 8x60 oligonucleotide ISCA array	NO	TOP
16	20	VSD	nasal bone agenesis	4p16.3p16.2(12241_3673850)x3, 9q34.3(138388970_141122255)x1	derivative translocation t(4p16.3p16.2;9q34.3)		de novo	High resolution 8x60 oligonucleotide ISCA array	NO	TOP
17	22	VSD	micro-retrognathia	4q32.2q35.1(162364653_185197402)x1	4q33 deletion syndrome	4q33 deletion syndrome		High resolution 8x60 oligonucleotide ISCA array	YES	TOP
18	21.1	interrupted aortic arch	isolated	22q11.21(18765102_20311733)x1	Proximal 22q11.2 deletion (classic, LCR22A-LCR22D) (DiGeorge/velocardiofacial syndromes)	22q11.2 deletion syndrome	de novo	High resolution 8x60 oligonucleotide ISCA array	NO	FO: 3 meses, iatrogenic tracheomalacia
19	25.5	VSD	holoprosencephaly; fetal growth restriction	13q31.3_q34(92587094_115093115)x1	ZIC2	Deletion includes ZIC2 (MIM603073). Defects in ZIC2 cause holoprosencephaly		High resolution 8x60 oligonucleotide ISCA array	YES	TOP
20	14.1	Hypoplastic Right Heart Syndrome	bilateral lip/paladar cleft; Hidrothorax; fetal growth restriction; hyperechogenic kidney	9p24.3p13.1(204090_38815471)x3~4	9p mosaic tetrasomy syndrome +i(9)(p24p12)		de novo	High resolution 8x60 oligonucleotide ISCA array	YES	TOP
21	25	VSD	isolated	8q22.1(96187828_14636402)x3; 18qp11.32(1_14081934)x1	46,XY,der(18)t(8;18)(q22.1;p11.32)		paternal	High resolution 8x60 oligonucleotide ISCA array	YES	TOP
22	24	aortic arch hypoplasia		(15)x2~3	mosaic trisomy chromosome 15		de novo		YES	TOP
23	27	VSD	congenital pulmonary	7q31.1q31.31(112516443_	7q31.1q31.31 duplication	related to severe intellectual disability/ pulmonar sequestration	de novo	High resolution 8x60	NO	TOP

Case	Gestational age (weeks)	cardiac anomaly	Other anomalies	ISCN formula	Genetic content	Comments	De novo inherited	Test	visible by karyotype	Follow-up
			airway malformation	119186429)x3				oligonucleotide ISCA array		
24	21.5	Shone syndrome		7q11.23(72766313_74133332)x1	7q11.23 frequent deletion (95%) (Williams-Beuren syndrome, WBS)	Williams-Beuren syndrome	de novo	High resolution 8x60 oligonucleotide ISCA array	NO	TOP
25	21	pulmonary atresia	isolated	22q11.21(18894820_21457610)x1	Proximal 22q11.2 deletion (classic, LCR22A-LCR22D) (DiGeorge/velocardiofacial syndromes)	22q11.2 deletion syndrome	de novo	High resolution 8x60 oligonucleotide ISCA array	NO	TOP

6.4 STUDY 3: THORACIC ANOMALIES

Seventy-one cases of fetal thoracic anomalies in which an array-CGH study had been performed, were identified. None of these cases presented an abnormal QF-PCR. In 3 cases the array was run, but the genetic results were not available due to a technical failure. Therefore 68 cases were included in the final analysis.

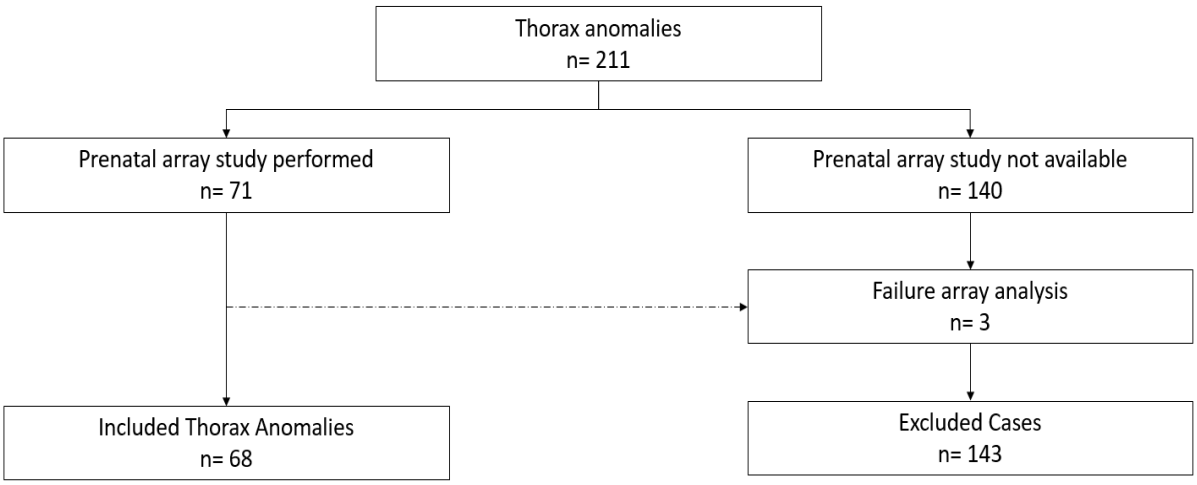


Figure 8: Thoracic anomalies study. Flowchart: selection criteria

Demographic characteristics

In the thoracic anomalies group the median maternal age was 32 years (IQR, 18 to 44), with a median gestational age at invasive testing of 21.5 weeks (IQR, 12.4 to 36.1). Thirty-eight women (55.9%) were nulliparous, and 30 (44.1%) were multiparous. Sixty-seven (98.5%) were singleton pregnancies, and one (1.5%) was a dichorionic twin pregnancy.

Type of anomaly

A thoracic anomaly was present as isolated in 38 (55.9%) out of the 68 cases, while one or more associated anomalies were identified in the remaining 30 (44.1%) cases.

Fetal growth restriction was diagnosed in 6 (8.8%) cases. All cases with a FGR also presented other major abnormalities.

Thoracic anomalies were divided into subgroups as follows: chest wall deformities (n=5, 7.4%); congenital diaphragmatic hernia (n=30, 44.1%); hydrothorax (n=23, 33.8%); pulmonary hypoplasia (n=1, 1.5%) and thoracic mass (n=9, 13.2%).

In the isolated thoracic anomalies group (n=38) the anomalies found were chest wall deformities (n=1, 2.6%), congenital diaphragmatic hernia (n=21, 55.2%); hydrothorax (n=10, 26.3%); pulmonary hypoplasia (n=1, 2.6%) and thoracic mass(n=5, 13.2%).

In the group with associated extra thoracic anomalies, the abnormalities diagnosed were chest wall deformities (n=4, 12.9%), congenital diaphragmatic hernia (n=9, 29%); hydrothorax (n=13, 41.9 %); thoracic mass (n=4, 12.9%). No cases of pulmonary hypoplasia were found in this group.

Array study

In 61 (89.7 %) cases, the invasive test performed was an amniocentesis, in 6 (8.8%) cases a chorionic villous sampling was performed and in 1 (1.5 %) case, a fetal sample tissue for chromosomal analysis was obtained following a termination of pregnancy.

Pathogenic CNVs were diagnosed in 7 cases (10.3 %) and VUS were found in 4 cases (5.9%); a normal array was present in 57 (83.8 %) cases

Table 11 shows pathogenic CNV and VUS depending on the main type and the subgroup of thoracic anomaly detected, either isolated or associated with other anomalies, respectively.

When only isolated thoracic anomalies were considered, in 3 of the 38 (7.9%) cases, a pathogenic CNV was found; conversely, in 4 of the 30 (13.3%) cases of thoracic anomaly with one or more associated major anomaly and associated FGR, a pathogenic CNV was diagnosed.

A VUS was found in 2 of the 38 (5.3%) cases with an isolated thoracic anomaly and in 2 (6.7%) of the 30 cases with associated major anomalies and FGR.

Table 12 reports the description of each case with pathogenic CNV

Table 11. Prevalence of pathogenic copy number variants (CNVs) and variants of unknown significance (VUS) in the different types of thoracic anomalies.

	ALL(n=68)			ISOLATED(n=38)			ASSOCIATED(n=30)		
	N	Pathogenic	VUS	N	Pathogenic	VUS	N	Pathogenic	VUS
CHEST WALL DEFORMITIES	5/68 (7.35%)	0/5 (0%)	0/5 (0%)	1/38 (2.63%)	0/1 (0%)	0/1 (0%)	4/30 (13.3%)	0/4 (0%)	0/4 (0%)
ectopia cordis	1/5 (20%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/0 (0%)	0/0 (0%)	1/4 (25%)	0/1 (0%)	0/1 (0%)
fused ribs	1/5 (20%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/0 (0%)	0/0 (0%)	1/4 (25%)	0/1 (0%)	0/1 (0%)
hypoplastic thorax	3/5 (60%)	0/3 (0%)	0/3 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	2/4 (50%)	0/2 (0%)	0/2 (0%)
CDH	30/68 (44.1%)	2/30 (6.66%)	1/30 (3.33%)	21/38 (55.2%)	1/21 (4.76%)	1/21 (4.76%)	9/30 (30%)	1/9 (11.1%)	0/9 (0%)
CDH-LCDH	24/30 (80%)	2/24 (8.33%)	1/24 (4.16%)	19/21 (90.4%)	1/19 (5.26%)	1/19 (5.26%)	5/9 (55.5%)	1/5 (20%)	0/5 (0%)
CDH-RCDH	6/30 (20%)	0/6 (0%)	0/6 (0%)	2/21 (9.52%)	0/2 (0%)	0/2 (0%)	4/9 (44.4%)	0/4 (0%)	0/4 (0%)
HIDROTORAX	23/68 (33.8%)	4/23 (17.3%)	3/23 (13.0%)	10/38 (26.3%)	2/10 (20%)	1/10 (10%)	13/30 (43.3%)	2/13 (15.3%)	2/13 (15.3%)
hydrothorax	23/23 (100%)	4/23 (17.3%)	3/23 (13.0%)	10/10 (100%)	2/10 (20%)	1/10 (10%)	13/13 (100%)	2/13 (15.3%)	2/13 (15.3%)
PULMONARY HYPOPLASIA	1/68 (1.47%)	0/1 (0%)	0/1 (0%)	1/38 (2.63%)	0/1 (0%)	0/1 (0%)	0/30 (0%)	0/0 (0%)	0/0 (0%)
pulmonary agenesis	1/23 (4.34%)	0/1 (0%)	0/1 (0%)	1/10 (10%)	0/1 (0%)	0/1 (0%)	0/13 (0%)	0/0 (0%)	0/0 (0%)
THORACIC MASS	9/68 (13.2%)	1/9 (11.1%)	0/9 (0%)	5/38 (13.1%)	0/5 (0%)	0/5 (0%)	4/30 (13.3%)	1/4 (25%)	0/4 (0%)
teratoma	1/9 (11.1%)	0/1 (0%)	0/1 (0%)	1/5 (20%)	0/1 (0%)	0/1 (0%)	0/4 (0%)	0/0 (0%)	0/0 (0%)
congenital pulmonary airway malf	5/9 (55.5%)	1/5 (20%)	0/5 (0%)	3/5 (60%)	0/3 (0%)	0/3 (0%)	2/4 (50%)	1/2 (50%)	0/2 (0%)
pulmonary sequestration	3/9 (33.3%)	0/3 (0%)	0/3 (0%)	1/5 (20%)	0/1 (0%)	0/1 (0%)	2/4 (50%)	0/2 (0%)	0/2 (0%)

Table 12. Details of all cases with pathogenic CNVs in the different types of thoracic anomalies.

Case	Gestational age (weeks)	cardiac anomaly	Other anomalies	ISCN formula	Genetic content	Comments	De novo inherited	Test	visible by karyotype	Follow-up
1	16.2	cervical lymphangiomas	ventriculomegaly,renal cyst, LCDH	Xq26.2(132315039_132876911)x0	Simpson-Golabi-Behmel syndrome type 1 (SGBS1; MIM:312870, ORPHA:373)	Ventriculomegaly, congenital diaphragmatic hernia, renal cyst: Simpson-Golabi-Behmel Syndrome Type 1. Genes, GPC4 (OMIM: 300168), GPC3 (OMIM: 300037)	maternal	High resolution 8x60 oligonucleotide ISCA array)	NO	TOP
2	17.3	ascites	Pulmonary stenosis, hydrothorax hyperechogenic kidneys; micrognathia	9p24.3p13.1(204090_38704041)x1	9p24.3p13.1 terminal deletion	Síndromes de deleción 9p24.1p22.3 y 9p13. DMRT1 DOCK8	de novo	High resolution 8x60 oligonucleotide ISCA array	YES	TOP
3	12.4	hidrothorax	isolated	Xp22.3q21.1(76117_74790543)x1~1.25, Xq21.1q26.3 (74900276_136418718) x1.25~1.5, Xq26.3q28(136470885_155246643)x1	Estructural reorganization chromosome X		de novo	High resolution 8x60 oligonucleotide ISCA array)	YES	stillbirth
4	25	left CDH	isolated	Xq26.2(132669976_133072947)x0 PATOGENICA, Xq21.1(78102922_79220332)x0 INCIERTA	GPC3. simpson-golabi-behmel syndrome	GPC3. simpson-golabi-behmel syndrome	de novo	High resolution 8x60 oligonucleotide ISCA array)	NO	TOP
5	20	hidrothorax	isolated	5p15.33p15.31(78947 _7458976)x1, 15q26.3(100923576_102383614)x3, 15q11.2(22648239_23217655)x1	5p15.33p15.31 terminal deletion (Cri du chat)	Cri du chat	paternal	High resolution 8x60 oligonucleotide ISCA array)	YES (5p15.33p15.31) NO (15q26.3 and 15q11.2)	TOP
6	14.1	hidrothorax	Hypoplastic Right Heart Syndrome,bilateral lip/paladar cleft; ; fetal growth restriction; hyperechogenic kidney	9p24.3p13.1(204090_38815471)x3~4	9p mosaic tetrasomy syndrome +i(9)(p24p12)		de novo	High resolution 8x60 oligonucleotide ISCA array)	YES	TOP
7	27	congenital pulmonary airway malformation	VSD	7q31.1q31.31(112516443_119186429)x3	7q31.1q31.31 duplication	not known CNV but related to severe intellectual disability/ pulmonar sequestration	de novo	High resolution 8x60 oligonucleotide ISCA array)	NO	TOP

6.5 STUDY 4: GASTROINTESTINAL ANOMALIES

Nighty-eight cases with gastro-intestinal anomalies in which an array study had been performed were detected. From these, nine cases were excluded: 4 cases presented an abnormal QF-PCR (one case of trisomy 21, one trisomy 13, one trisomy 18 and one case of trisomy 9), in three cases a fetal infection that could explain the ultrasound findings was detected (2 cases of cytomegalovirus and one cases of human herpes virus), and in 2 cases a failure in the array analysis occurred. Eighty-nine cases were included in the final analysis of gastrointestinal anomalies.

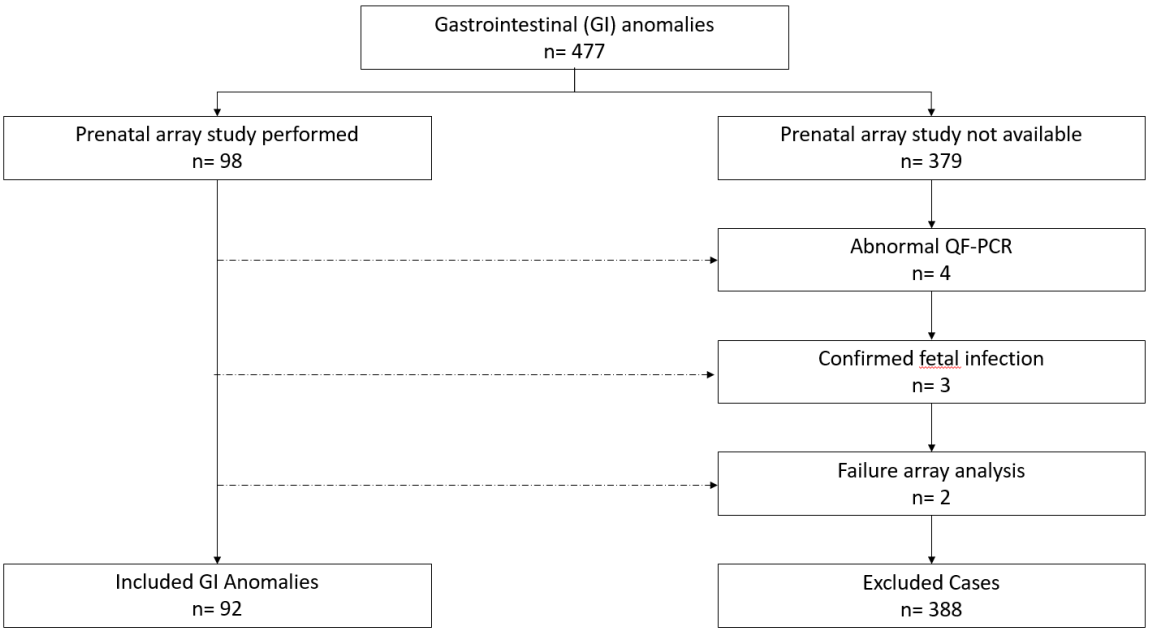


Figure 9: Gastrointestinal anomalies study. Flowchart: selection criteria

Demographic characteristics

The median maternal age was 32 years (IQR, 16 to 46); at invasive testing the median gestational age was 21.6 weeks (IQR, 11.4 to 33).

Forty-nine (55.1%) patients were nulliparous, while 40 (44.9%) were multiparous. Referring to the type of gestation, 82 (92.1%) were singleton pregnancies, 3 (3.4%) were dichorionic twin pregnancies, 2 (2.2%) were monochorionic twin pregnancies, and 2 (2.2%) were triplets.

Type of anomaly

An isolated gastrointestinal anomaly was detected in 47 (52.8%) out of the 89 cases, and one or more associated anomalies were detected in 42 (47.2%) out of the 89 cases. Associated fetal growth restriction was diagnosed in 10 (11.2%) cases, in all these cases the fetal growth alteration was present together with one or more other major anomaly. The remaining 79 (88.8%) cases did not present an association with FGR.

Gastrointestinal anomalies were divided into the following groups of anomalies: 46 (51.7%) cases of intestinal anomalies, 18 cases (20.2%) of hyperechogenic bowel, 13 (14.6%) cases of abdominal wall defects, 9 cases (10.1%) of hepatic anomalies, 2 cases (2.2%) of situs anomalies, and one case (1.1%) of pancreatic dysplasia.

In the group of the 47 isolated findings, gastrointestinal anomalies were divided as follows: hepatic anomalies (n=8, 17%), intestinal anomalies (n=22, 46.8%), hyperechogenic bowel (n=8, 17%), abdominal wall defects (n=8, 17%), situs anomaly (n=1, 2.1%), no cases of pancreatic dysplasia were present as isolated anomalies.

In the group with other associated anomalies (n=42 47.2%), the abnormalities diagnosed were: hepatic anomalies (n=1, 2.4%), intestinal anomalies (n=24, 57.1%), hyperechogenic bowel (n=10, 23.8%), abdominal wall defects (n=5, 11.9%), situs anomaly (n=1, 2.4 %), and one case (2.4 %) of pancreatic dysplasia.

Array study

In 81 (91.0%) cases chromosomal diagnosis was achieved following an amniocentesis, in 7 (7.9%) cases a CVS was performed, and in one case (1.1%) genetic testing was performed on fetal tissue after a termination of pregnancy.

Pathogenic CNVs were found in 5 (5.6%) cases, VUS were found in 5 (5.6%) cases, while a normal result was obtained in 79 (88.8%) cases.

In **Table 13,** pathogenic CNVs and VUS for each of the subgroups of gastrointestinal anomalies are shown, isolated or associated with other anomalies, respectively.

If only isolated anomalies are considered, in 1 out of the 47 cases (2.1%), a pathogenic CNV was found; conversely, in the group of gastrointestinal anomalies with one or more associated anomalies in 4 out of the 42 (9.5%) cases, a pathogenic CNV is diagnosed.

A variant of unknown significance was found in 2 of the 47 (4.3%) cases with an isolated gastrointestinal anomaly, and in 3 (7.1%) of the 42 cases with associated major anomalies and fetal growth restriction.

Table 14 reports the description of each case with pathogenic CNV.

Table 13. Prevalence of pathogenic copy number variants (CNVs) and variants of unknown significance (VUS) in the different types of gastrointestinal anomalies.

	ALL(n=89)			ISOLATED(n=47)			ASSOCIATED(n=42)		
	N	Pathogenic	VUS	N	Pathogenic	VUS	N	Pathogenic	VUS
hepatic anomalies	9/89 (10.1%)	0/9 (0%)	0/9 (0%)	8/47 (17.0%)	0/8 (0%)	0/8 (0%)	1/42 (2.3%)	0/1 (0%)	0/1 (0%)
hepatic calcifications	8/9 (88.8%)	0/8 (0%)	0/8 (0%)	7/8 (87.5%)	0/7 (0%)	0/7 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
hepatomegaly	1/9 (11.1%)	0/1 (0%)	0/1 (0%)	1/8 (12.5%)	0/1 (0%)	0/1 (0%)	0/83 (0%)	0/0 (0%)	0/0 (0%)
hyperechogenicity	18/89 (20.2%)	2/18 (11.1%)	0/18 (0%)	8/47 (17.0%)	0/8 (0%)	0/8 (0%)	10/42 (23.8%)	2/10 (20%)	0/10 (0%)
hyperechogenic bowel	18/18 (100%)	2/18 (11.1%)	0/18 (0%)	8/8 (100%)	0/8 (0%)	0/8 (0%)	10/10 (100%)	2/10 (20%)	0/10 (0%)
Intestinal anomalies	46/89 (51.6%)	2/46 (4.34%)	3/46 (6.52%)	22/47 (46.8%)	0/22 (0%)	1/22 (4.5%)	24/42 (57.1%)	2/24 (8.33%)	2/24 (8.33%)
anal malformation	1/46 (2.1%)	0/1 (0%)	0/1 (0%)	0/22 (0%)	0/0 (0%)	0/0 (0%)	1/24 (4.16%)	0/1 (0%)	0/1 (0%)
duodenal atresia	2/46 (4.3%)	0/2 (0%)	0/2 (0%)	1/22 (4.5%)	0/1 (0%)	0/1 (0%)	1/24 (4.16%)	0/1 (0%)	0/1 (0%)
esophagus atresia	9/46 (19.5%)	1/9 (11.1%)	0/9 (0%)	5/22 (22.7%)	0/5 (0%)	0/5 (0%)	4/24 (16.6%)	1/4 (25%)	0/4 (0%)
ascites	13/46 (28.2%)	1/13 (7.69%)	2/13 (15.3%)	2/22 (9.0%)	0/2 (0%)	0/2 (0%)	11/24 (45.8%)	1/11 (9.0%)	2/11 (18.1%)
rectal atresia	1/46 (2.1%)	0/1 (0%)	0/1 (0%)	0/22 (0%)	0/0 (0%)	0/0 (0%)	1/24 (4.16%)	0/1 (0%)	0/1 (0%)
intestinal calcifications	7/46 (15.2%)	0/7 (0%)	0/7 (0%)	4/22 (18.1%)	0/4 (0%)	0/4 (0%)	3/24 (12.5%)	0/3 (0%)	0/3 (0%)
bowel dilatation	3/46 (6.5%)	0/3 (0%)	0/3 (0%)	2/22 (9.0%)	0/2 (0%)	0/2 (0%)	1/24 (4.16%)	0/1 (0%)	0/1 (0%)
bowel obstruction	5/46 (10.8%)	0/5 (0%)	1/5 (20%)	4/22 (18.1%)	0/4 (0%)	1/4 (25%)	1/24 (4.16%)	0/1 (0%)	0/1 (0%)
cyst	5/46 (10.8%)	0/5 (0%)	0/5 (0%)	4/22 (18.1%)	0/4 (0%)	0/4 (0%)	1/24 (4.16%)	0/1 (0%)	0/1 (0%)

	ALL(n=89)			ISOLATED(n=47)			ASSOCIATED(n=42)		
	N	Pathogenic	VUS	N	Pathogenic	VUS	N	Pathogenic	VUS
Pancreatic anomalies	1/89 (1.1%)	0/1 (0%)	0/1 (0%)	0/47 (0%)	0/0 (0%)	0/0 (0%)	1/42 (2.3%)	0/1 (0%)	0/1 (0%)
pancreatic dysplasia	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
abdominal wall	13/89 (14.6%)	1/13 (7.69%)	1/13 (7.69%)	8/47 (17.0%)	1/8 (12.5%)	1/8 (12.5%)	5/42 (11.9%)	0/5 (0%)	0/5 (0%)
other abdominal wall defects	1/13 (7.6%)	0/1 (0%)	0/1 (0%)	0/8 (0%)	0/0 (0%)	0/0 (0%)	1/5 (20%)	0/1 (0%)	0/1 (0%)
gastroschisis	3/13 (23.0%)	0/3 (0%)	0/3 (0%)	2/8 (25%)	0/2 (0%)	0/2 (0%)	1/5 (20%)	0/1 (0%)	0/1 (0%)
omphalocele	8/13 (61.5%)	1/8 (12.5%)	1/8 (12.5%)	5/8 (62.5%)	1/5 (20%)	1/5 (20%)	3/5 (60%)	0/3 (0%)	0/3 (0%)
teratoma	1/13 (7.6%)	0/1 (0%)	0/1 (0%)	1/8 (12.5%)	0/1 (0%)	0/1 (0%)	0/5 (0%)	0/0 (0%)	0/0 (0%)
situs anomalies	2/89 (2.2%)	0/2 (0%)	1/2 (50%)	1/47 (2.1%)	0/1 (0%)	0/1 (0%)	1/42 (2.3%)	0/1 (0%)	1/1 (100%)
isomerism	1/2 (50%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/0 (0%)	0/0 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)
situs inversus	1/2 (50%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/0 (0%)	0/0 (0%)

Table 14. Details of all cases with pathogenic CNVs in the different types of gastrointestinal anomalies.

Case	Gestational age (weeks)	cardiac anomaly	Other anomalies	ISCN formula	Genetic content	Comments	De novo inherited	Test	visible by karyotype	Follow-up
1	21	hyperechogenic bowel	talipes	16p12.2(21631626_21728426)x0	Deafness, autosomal recessive 22 (MIM: 607039)	OTOA	not studied	High resolution 8x60 oligonucleotide ISCA array)	NO	no follow up
2	16	omphalocele		8q24.3(142582952_144115101)x1	One patient with developmental behavioral abnormalities and dysmorphic features (De-novo interstitial 2.33 Mb deletion in 8q24.3: new insights on a very rare partial monosomy syndrome)		not studied	High resolution 8x60 oligonucleotide ISCA array)	NO	TOP
3	17.3	ascites	Pulmonary stenosis, hydrothorax hyperechogenic kidneys; micrognathia	9p24.3p13.1(204090_38704041)x1	9p24.3p13.1 terminal deletion	deletion syndromes 9p24.1p22.3 and 9p13. DMRT1 DOCK8	de novo	High resolution 8x60 oligonucleotide ISCA array	YES	TOP
4	27.4	hyperechogenic bowel	corpus callosum agenesis, FGR, skeletal anomalies	1q43q44(241293508_249203359)x1	1q43q44 deletion		not studied	High resolution 8x60 oligonucleotide ISCA array	NO	TOP
5	23.6	esophagic atresia	micro retrognathia	14q11.2q21.3 (20608211_49914251)x3, 21q11.2q22.11(15502350_34091304)x1	derivative translocation t(14q11.2q21.3;21q11.2q22.11). Proximal duplication 14q12 including the FOXG1 (MIM:164874). 21q21.1 deletion (NCAM2)	21q21.1 deletion (NCAM2) is associated with developmental delay, speech defect, behavioural problems, autism spectrum disorder (ASD) and a large head circumference[218].- Proximal duplication 14q12 including the FOXG1 (MIM:164874) show phenotype that resembles the FOXG1 haploinsufficiency syndrome. Described phenotype in 14 reported patients is characterized by non specific dysmorphic features, epilepsy (with onset within the first year of life). Depending on the size of the duplication and the included genes within the region, clinical features may vary between patients [219]. Patients with deletions and inactivating mutations within the chromosomal region 14q12, including the FOXG1 gene, have similar clinical features such as developmental delay, hand stereotypies, deceleration of head growth, and epilepsy. However, patients with duplications may be differentiated from those with deletions or inactivating mutations based on	maternal	High resolution 8x60 oligonucleotide ISCA array)	YES	TOP

Case	Gestational age (weeks)	cardiac anomaly	Other anomalies	ISCN formula	Genetic content	Comments	De novo inherited	Test	visible by karyotype	Follow-up
						certain clinical aspects, such as the ability to walk within two years, severe speech disabilities, earlier seizure onset (seizure onset within the first years of life), the absence of the stereotypic hand movements, and significant microcephaly. Variable penetrance or lack of association between FOXG1 duplication and phenotypic abnormalities has been suggested.[220] It has been proposed that duplications in afected patients include a putative cis-regulatory element distal to FOXG1 gene in 14 29118250 29147249 and normal carriers not.				

6.6 STUDY 5: FACIAL ANOMALIES

Sixty-eight cases with facial anomalies and array-CGH study were found. One case with abnormal QFPCR was identified (1 case of Turner syndrome), and in another case the array analysis was run but ended up in a failure with no results available; therefore 66 cases were included for the final analysis.

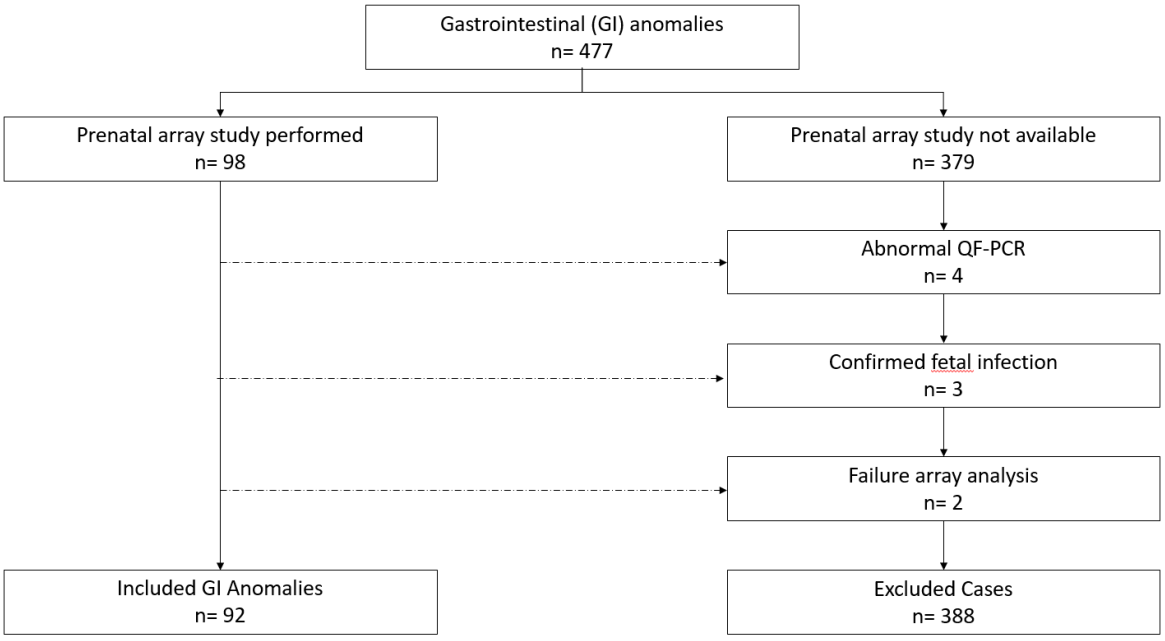


Figure 10: Gastrointestinal anomalies study. Flowchart: selection criteria

Demographic characteristics

The median maternal age for this group was 32 years (IQR, 18 to 42). The median gestational age at invasive testing was 20.6 weeks (IQR, 12 to 35). Thirty-nine women (59.1%) were nulliparous, while 27 (40.9 %) were multiparous. Sixty (90.9%) were singleton pregnancies, 3 (4.5%) were dichorionic twin pregnancies, 2 (3.0%) were monochorionic twin pregnancies, and one case (1.5%) was a triplet.

Type of anomaly

The facial anomaly was present as isolated in 22 (33.3%) out of the 66 cases; one or more associated major abnormalities were found in 44 (66.7%) out of the total cases.

Fetal growth restriction was diagnosed in 10 (15.2%) cases, the remaining 56 cases (84.8%) did not present an associated fetal growth restriction. In all cases in which an FGR was identified, other major abnormalities were identified.

The facial anomalies identified were divided as follows: mouth and lips anomalies (n=21, 31.8%); face and profile anomalies (n=19 28.8%); subcutaneous edema (n=1, 1.5%); cervical anomalies (n=13, 19.7%); nose anomalies (n=4, 6.1%); eye anomalies (n=4, 6.1%), and external ear anomalies (n=4, 6.1%).

In the isolated group (n=22), the facial anomalies found were 14 mouth/lips anomalies 63.6%), 5 cases (22.7%) of cervical anomalies and 3 cases (13.6%) of face/profile anomalies.

In the group of 44 cases with associated extra facial anomalies, the abnormalities diagnosed were: 7 cases of mouth/lips anomalies (15.9%), 1 case of subcutaneous edema (2.3%), 8 cases (18.2%) of cervical anomalies, 4 cases each (9.1%) of nose abnormalities, eye and external ear anomalies. Finally, 16 cases (36.4%) of face/profile anomalies were identified in this group.

Array study

Considering the type of invasive test, in 54 cases (81.8%), an amniocentesis was performed, in 9 (13.6%) cases, results were obtained following a chorionic villus

sampling, and in 3 cases (4.5%) fetal cell for the array and chromosomal analysis were obtained with direct biopsy after a termination of pregnancy.

Pathogenic CNVs were diagnosed in 11 (16.7%) cases, variants of unknown significance in 4 cases (6.1%) and normal results were obtained in 51 cases (77.3%).

Table 15 shows pathogenic CNVs and VUS CNVs for facial anomalies either isolated or associated with other abnormalities.

In the subgroup with isolated anomalies, no pathogenic CNV was found, while in the group with associated anomalies, 11 (25%) pathogenic CNVs were diagnosed. No VUS was found in the isolated anomalies group compared with 4 (9.1%) out of the 44 cases with associated anomalies.

Among the 44 cases of facial anomalies with extra facial associated abnormalities, 10 cases of fetal growth restriction were found, with the following results regarding array study: 3 (30%) cases of pathogenic CNVs, 1 (10%) case of a variant of unknown significance and in the remaining cases (n=6, 60%) of fetal growth restriction the array study ended up in a normal result.

Table 16 reports the description of each case with pathogenic CNV

Table 15. Prevalence of pathogenic copy number variants (CNVs) and variants of unknown significance (VUS) in the different types of facial anomalies.

	ALL(n=66)			ISOLATED(n=22)			ASSOCIATED(n=44)		
	N	Pathogenic	VUS	N	Pathogenic	VUS	N	Pathogenic	VUS
Mouth/lips anomalies	21/66 (31.8%)	2/21 (9.52%)	1/21 (4.76%)	14/22 (63.6%)	0/14 (0%)	0/14 (0%)	7/44 (15.9%)	2/7 (28.5%)	1/7 (14.2%)
lip/palate bilateral cleft	8/21 (38.0%)	2/8 (25%)	0/8 (0%)	4/14 (28.5%)	0/4 (0%)	0/4 (0%)	4/7 (57.1%)	2/4 (50%)	0/4 (0%)
lip/palate central cleft	1/21 (4.76%)	0/1 (0%)	0/1 (0%)	1/14 (7.14%)	0/1 (0%)	0/1 (0%)	0/7 (0%)	0/0 (0%)	0/0 (0%)
lip/palate unilateral cleft	12/21 (57.1%)	0/12 (0%)	1/12 (8.33%)	9/14 (64.2%)	0/9 (0%)	0/9 (0%)	3/7 (42.8%)	0/3 (0%)	1/3 (33.3%)
Face anomalies	1/66 (1.51%)	0/1 (0%)	1/1 (100%)	0/22 (0%)	0/0 (0%)	0/0 (0%)	1/44 (2.27%)	0/1 (0%)	1/1 (100%)
subcutaneous edema	1/8 (12.5%)	0/1 (0%)	1/1 (100%)	0/4 (0%)	0/0 (0%)	0/0 (0%)	1/4 (25%)	0/1 (0%)	1/1 (100%)
Cervical anomalies	13/66 (19.6%)	2/13 (15.3%)	0/13 (0%)	5/22 (22.7%)	0/5 (0%)	0/5 (0%)	8/44 (18.1%)	2/8 (25%)	0/8 (0%)
cystic hygroma	6/13 (46.1%)	1/6 (16.6%)	0/6 (0%)	0/5 (0%)	0/0 (0%)	0/0 (0%)	4/8 (50%)	1/4 (25%)	0/4 (0%)
cervical lymphangiomas	5/13 (38.4%)	1/5 (20%)	0/5 (0%)	3/5 (60%)	0/3 (0%)	0/3 (0%)	2/8 (25%)	1/2 (50%)	0/2 (0%)
cervical tumor	1/13 (7.69%)	0/1 (0%)	0/1 (0%)	0/5 (0%)	0/0 (0%)	0/0 (0%)	1/8 (12.5%)	0/1 (0%)	0/1 (0%)
nuchal edema	1/13 (7.69%)	0/1 (0%)	0/1 (0%)	2/5 (40%)	0/2 (0%)	0/2 (0%)	1/8 (12.5%)	0/1 (0%)	0/1 (0%)
Nose anomalies	4/66 (6.06%)	1/4 (25%)	0/4 (0%)	0/22 (0%)	0/0 (0%)	0/0 (0%)	4/44 (9.09%)	1/4 (25%)	0/4 (0%)
nasal bone agenesis	3/4 (75%)	1/3 (33.3%)	0/3 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)	3/4 (75%)	1/3 (33.3%)	0/3 (0%)
hypoplastic nose	1/4 (25%)	0/1 (0%)	0/1 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)	1/4 (25%)	0/1 (0%)	0/1 (0%)
Ocular anomalies	4/66 (6.06%)	1/4 (25%)	0/4 (0%)	0/22 (0%)	0/0 (0%)	0/0 (0%)	4/44 (9.09%)	1/4 (25%)	0/4 (0%)

	ALL(n=66)			ISOLATED(n=22)			ASSOCIATED(n=44)		
	N	Pathogenic	VUS	N	Pathogenic	VUS	N	Pathogenic	VUS
exophthalmos	1/4 (25%)	0/1 (0%)	0/1 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)	1/4 (25%)	0/1 (0%)	0/1 (0%)
hypertelorism	2/4 (50%)	1/2 (50%)	0/2 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)	2/4 (50%)	1/2 (50%)	0/2 (0%)
eyelashes anomalies	1/4 (25%)	0/1 (0%)	0/1 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)	1/4 (25%)	0/1 (0%)	0/1 (0%)
External ear anomalies	4/66 (6.06%)	1/4 (25%)	1/4 (25%)	0/22 (0%)	0/0 (0%)	0/0 (0%)	4/44 (9.09%)	1/4 (25%)	1/4 (25%)
hypoplastic external ear	1/4 (25%)	0/1 (0%)	0/1 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)	1/4 (25%)	0/1 (0%)	0/1 (0%)
implantation anomalies	3/4 (75%)	1/3 (33.3%)	1/3 (33.3%)	0/0 (0%)	0/0 (0%)	0/0 (0%)	3/4 (75%)	1/3 (33.3%)	1/3 (33.3%)
Face and profile anomalies	19/66 (28.7%)	4/19 (21.0%)	1/19 (5.26%)	3/22 (13.6%)	0/3 (0%)	0/3 (0%)	16/44 (36.3%)	4/16 (25%)	1/16 (6.25%)
dysmorphic face	1/19 (5.26%)	0/1 (0%)	0/1 (0%)	0/3 (0%)	0/0 (0%)	0/0 (0%)	1/16 (6.25%)	0/1 (0%)	0/1 (0%)
other profile anomalies	2/19 (10.5%)	0/2 (0%)	0/2 (0%)	2/3 (66.6%)	0/2 (0%)	0/2 (0%)	15/16 (93.7%)	4/15 (26.6%)	1/15 (6.66%)
micro retrognathia	16/19 (84.2%)	4/16 (25%)	1/16 (6.25%)	1/3 (33.3%)	0/1 (0%)	0/1 (0%)	0/16 (0%)	0/0 (0%)	0/0 (0%)

Table 16. Details of all cases with pathogenic CNVs in the different types of facial anomalies.

Case	Gestational age (weeks)	cardiac anomaly	Other anomalies	ISCN formula	Genetic content	Comments	De novo inherited	Test	visible by karyotype	Follow-up
1	20	micro retrognathia	pelvic kidney	4p16.3p15.33 (91545_14988069)x1	4p16.3 terminal deletion (Wolf-Hirschhorn syndrome)	Wolf-Hirschhorn syndrome	de novo	High resolution 8x60 oligonucleotide ISCA array	YES	TOP
2	16.2	cervical lymphangiomas	ventriculomegaly,renal cyst, LCDH	Xq26.2(132315039_132876911)x0	Simpson-Golabi-Behmel syndrome type 1 (SGBS1; MIM:312870, ORPHA:373)	Ventriculomegaly, congenital diaphragmatic hernia, renal cyst: Simpson-Golabi-Behmel Syndrome Type 1. Genes, GPC4 (OMIM: 300168), GPC3 (OMIM: 300037)	maternal	High resolution 8x60 oligonucleotide ISCA array	NO	TOP
3	18	hyperteloris m	Dandy Walker malformation, Hypoplastic left heart with double outlet right ventricle; Cystic higroma	1q21.1q21.2(145415190_147380935)x3	1q21.1 proximal duplication TAR region (RBM8A) + 1q21.1 distal duplication (BP3-BP4, GJA5)	Complete 1q21.1 duplication (proximal + distal). q21 distal (BP3-BP4) duplication show variable penetrance of 17-47% and its known to be associated to brain and heary anomalies	paternal	High resolution 8x60 oligonucleotide ISCA array)	NO	TOP
4	15	lip/palate bilateral cleft	Abnormal sulcation	1p36.33p36.32(794596_4458182)x1	1p36 deletion syndrome	Brain anomalies and orofacial clefting are part of the 1p36 deletion syndrome	de novo	Low resolution BAC array	NO	TOP
5	13.1	cystic hygroma	Hypoplastic Left Heart Syndrome	4p16.3p15.1(75647_35238188)x1, 4p15.2p15.1(25428634_33308093)x1	4p16.3 deletion (Wolf-Hirschhorn syndrome)		de novo	High resolution 8x60 oligonucleotide ISCA array)	YES	TOP
6	24	implantation anomalies	Ductus venosus agenesis	3p25.2 x1	RAF1 gene		not studied	High resolution 8x60 oligonucleotide ISCA array)	NO	Neonatal death
7	20	nasal bone agenesis	ventricular septal defect	4p16.3p16.2(12241_3673850)x3, 9q34.3(138388970_141122255)x1	derivative translocation t(4p16.3p16.2;9q34.3)		de novo	High resolution 8x60 oligonucleotide ISCA array)	NO	TOP
8	22	microretrognathia	ventricular septal defect	4q32.2q35.1(162364653_185197402)x1	4q33 deletion syndrome	Region 4q33 has been proposed as the critical region for 4q deletion syndrome.[221] Almost all individuals with 4q deletions have craniofacial and digital anomalies, more than half had skeletal anomalies, and almost half congenital heart disease (CHD). Autistic spectrum disorder and attention deficit hyperactivity disorder are part of the behavioral phenotype in 4q deletion syndrome. Interstitial deletions have been associated with short limbs and small hands, Rieger syndrome and		High resolution 8x60 oligonucleotide ISCA array)	YES	TOP

Case	Gestational age (weeks)	cardiac anomaly	Other anomalies	ISCN formula	Genetic content	Comments	De novo inherited	Test	visible by karyotype	Follow-up
						piebaldism, the more distal deletions involving 4q34-q35 with a lesser degree of characteristic features and cognitive impairment and terminal deletions involving 4q34 with satyr ears and hypoplastic fifth finger with a distinctive pointed nail				
9	14.1	lip/palate bilateral cleft	Hypoplastic Right Heart Syndrome, hyperechogenic kidney , hydrothorax, FGR	9p24.3p13.1(204090_38815471)x3~4	9p mosaic tetrasomy syndrome +i(9)(p24p12)	Clinical phenotype of tetrasomy 9p includes a variety of physical and developmental abnormalities. Commonly, patients have distinctive facial appearances with hypertelorism, cleft lip or palate, ear anomalies, and micrognathia. In addition, recurrent clinical features include developmental delay, central nervous system anomaly, limb defects, postnatal growth failure, congenital heart disease, renal anomalies, and short neck with excess nuchal skin [222]	de novo	High resolution 8x60 oligonucleotide ISCA array)	YES	TOP
10	23.6	micro retrognathia	esophagic atresia	14q11.2q21.3(20608211_49914251)x3, 21q11.2q22.11(15502350_34091304)x1	derivative translocation t(14q11.2q21.3;21q11.2q22.11). Proximal duplication 14q12 including the FOXG1 (MIM:164874). 21q21.1 deletion (NCAM2)	21q21.1 deletion (NCAM2) is associated with developmental delay, speech defect, behavioural problems, autism spectrum disorder (ASD) and a large head circumference[218].- Proximal duplication 14q12 including the FOXG1 (MIM:164874) show phenotype that resembles the FOXG1 haploinsufficiency syndrome. Described phenotype in 14 reported patients is characterized by non specific dysmorphic features, epilepsy (with onset within the first year of life). Depending on the size of the duplication and the included genes within the region, clinical features may vary between patients [219]. Patients with deletions and inactivating mutations within the chromosomal region 14q12, including the FOXG1 gene, have similar clinical features such as developmental delay, hand stereotypies, deceleration of head growth, and epilepsy. However, patients with duplications may be differentiated from those with deletions or inactivating mutations based on certain clinical aspects, such as the ability to walk within two years, severe speech disabilities, earlier seizure onset (seizure onset within the first years of life), the absence of the stereotypic hand movements, and significant microcephaly. Variable penetrance or lack of association between FOXG1 duplication and phenotypic abnormalities has been suggested.[220] It has been proposed that duplications in afected patients include a putative cis-regulatory element distal to FOXG1 gene in 14 29118250 29147249 and normal carriers not.	maternal	High resolution 8x60 oligonucleotide ISCA array)	YES	TOP

Case	Gestational age (weeks)	cardiac anomaly	Other anomalies	ISCN formula	Genetic content	Comments	De novo inherited	Test	visible by karyotype	Follow-up
11	21	micro retrognathia	pelvic kidney , FGR,cryptorchidism	7q11.23(72766313_74133332)x1	7q11.23 frequent deletion (95%) (Williams-Beuren syndrome, WBS)	Williams-Beuren syndrome (SW) is characterized by (1) a very characteristic face: flattened nasal bridge with a bulbous tip, large mouth with a wide everted lower lip, full cheeks, periorbital edema, epicanto and often stellar iris. With age, the face becomes narrower and features more prominent cardiovascular manifestations, which are characterized by arterial stenosis, including supravalvular aortic stenosis (SVAS) and peripheral pulmonary stenosis, and hypertension (40%-50%); developmental delay or mild to moderate mental retardation (75%); behavioral and cognitive abnormalities; endocrine system problems;connective tissue abnormalities, and renal anomalies and urinary tract defects, including hydronephrosis, kidney stones, renal agenesis, bladder diverticulum, voiding dysfunction, urinary tract infections, and undescended testis and hypospadias. Cardiovascular abnormalities occur in a large proportion (~ 50–80%) of patients and account for most morbidity and mortality. In approximately 25% of WS cases, the unaffected parent in whom the chromosome deletion originated has an inversion on chromosome 7 involving the WBSCR. Approximately 6% of the general population has this inversion polymorphism [223].		High resolution 8x60 oligonucleotide ISCA array)	NO	TOP
1	20	micro retrognathia	pelvic kidney	4p16.3p15.33 (91545_14988069)x1	4p16.3 terminal deletion (Wolf-Hirschhorn syndrome)	Wolf-Hirschhorn syndrome	de novo	High resolution 8x60 oligonucleotide ISCA array	YES	TOP

6.7 STUDY 6: URINARY ANOMALIES

Seventy cases of urologic anomalies were detected. From these, one case was excluded for presenting a pathologic QF-PCR (Trisomy 21), and 1 case was excluded due to a failure in the array analysis; therefore 68 cases with urinary anomalies in which an array analysis was performed were included in our study.

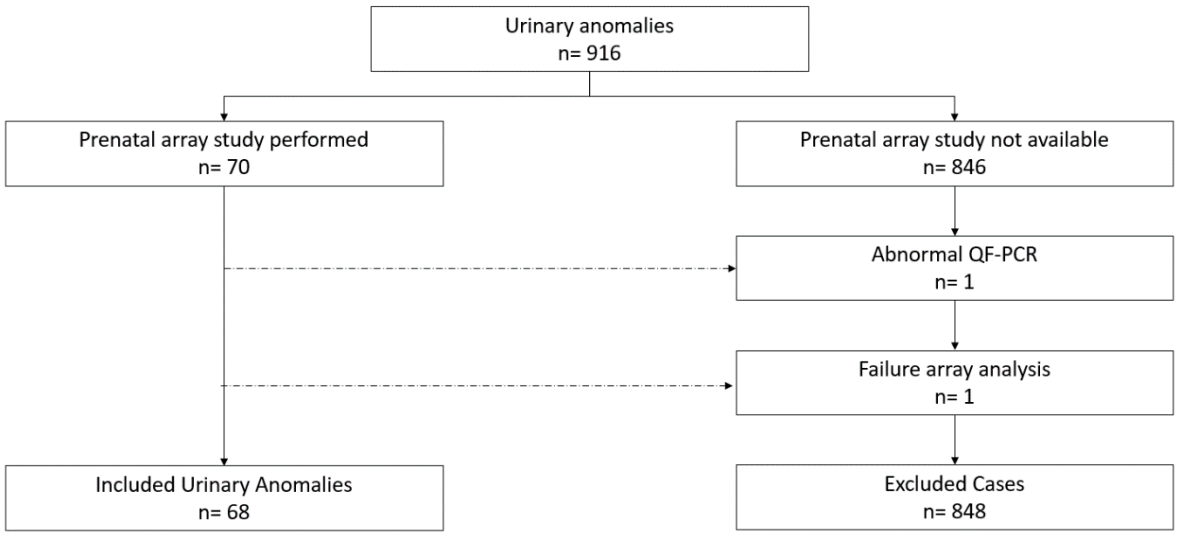


Figure 11: Urinary anomalies study. Flowchart: selection criteria

Demographic characteristics

In the urinary anomalies group the median maternal age found was 32 years (IQR, 18 to 45). The median gestational age at invasive testing was 21.0 weeks (IQR, 11 to 35). Thirty-eight (55.9%) women were nulliparous, and 30 (44.1%) were multiparous. Sixty (88.2%) were singleton pregnancies, 6 (8.8%) were dichorionic twin pregnancies, and 2 (2.9%) were monochorionic twin gestations.

Type of anomaly

The urologic anomalies were found to be isolated in 24 (35.3 %) cases while one or more associated major anomaly was found in 44 (64.7%) cases out of the 68 cases.

Fetal growth restriction was identified in 11 (16.2 %) cases, the remaining 57 cases (83.8 %) did not present associated fetal growth restriction. All the cases of anomalies with FGR were identified in the group of urologic anomalies with associated major abnormalities; conversely, no cases of FGR were found in the group of isolated urologic anomalies.

Anomalies of the urinary system were divided depending on the site and type of anomaly: for renal-ureteral anomalies the anomalies found were: anomalies of renal position (n=13, 19.1%), renal cystic diseases (n=11, 16.2%), urinary tract dilation (n=15 22.1%), duplicated collecting system (n=2, 2.9%), renal hyper echogenicity (n=5, 7.4%), renal malformation -including renal agenesis, hypoplasia, and dysplasia- (n=15, 22.1%); finally 7 cases (10.3%) of vesical anomalies were identified.

In the isolated urologic group (n=24) the anomalies identified were: 4 cases (16.7%) of kidney's position anomalies, 6 cases (25%) of renal cystic disease, 4 cases (16.7%) of urinary tract dilation, 2 cases (8.3%) of renal hyper echogenicity, 4 (16.7%) cases of renal malformation, and 4 cases (16.7%%) of vesical anomalies.

In the associated group (n=44), the following anomalies were diagnosed: 9 cases (20.4%) of kidney's position anomalies, 5 cases (11.4%) of renal cystic disease, 11 cases (25%) of urinary tract dilation, 2 cases (4.5%) of duplicated collecting system, 3 cases (6.8%) of

renal hyperechogenicity, 11 (25%) cases of renal malformation, and 3 cases (6.8%) of vesical anomalies.

Array study

Referring to the type of invasive test, in 53 cases (77.9%) an amniocentesis was performed; in 12 (17.6%) cases material was obtained from a chorionic villus sampling; in 1 case (1.5%) fetal cells for the chromosomal analysis were obtained from a fetal blood sample during a termination of pregnancy. Finally for 2 cases (2.9%) results were obtained from direct biopsy after a TOP. A pathogenic CNV was found in 6 out of the 68 cases (8.8%), a variant of unknown significance in 1 case (1.5 %); a normal result was found in 61 cases (89.7%). In Table 17 pathogenic CNVs and VUS result in case of urinary anomalies are shown, both isolated and associated to other extra urologic abnormalities. In the subgroup with isolated urologic anomalies, 1 (4.2%) pathogenic CNV was found, while in the group with associated anomalies 5 (11.4 %) pathogenic CNVs were identified. One case (4.2%) of variant of unknown significance was found in the group of isolated anomalies compared with no cases (0 %) of VUS among the 44 cases with associated anomalies. Among the 44 cases of urologic anomalies with others associated abnormalities, 11 cases of fetal growth restriction were found, with the following results for the array testing: 3 (27.3 %) cases of pathogenic CNVs, whereas no cases of variant of unknown significance were found in FGR subgroup; in the remaining 8 cases (72.7%) of urologic anomalies with associated fetal growth restriction the array study ended up in a normal result.

Table 18 reports the description of each case with pathogenic CNV

Table 17. Prevalence of pathogenic copy number variants (CNVs) and variants of unknown significance (VUS) in the different types of urinary anomalies.

	ALL(n=68)			ISOLATED(n=24)			ASSOCIATED(n=44)		
	N	Pathogenic	VUS	N	Pathogenic	VUS	N	Pathogenic	VUS
renal position anomalies	13/68 (19.1%)	2/13 (15.3%)	0/13 (0%)	4/24 (16.6%)	0/4 (0%)	0/4 (0%)	9/44 (20.4%)	2/9 (22.2%)	0/9 (0%)
horseshoe kidney	2/13 (15.3%)	0/2 (0%)	0/2 (0%)	1/4 (25%)	0/1 (0%)	0/1 (0%)	1/9 (11.1%)	0/1 (0%)	0/1 (0%)
pelvic kidney	11/13 (84.6%)	2/11 (18.1%)	0/11 (0%)	3/4 (75%)	0/3 (0%)	0/3 (0%)	8/9 (88.8%)	2/8 (25%)	0/8 (0%)
Cystic anomalies	11/68 (16.1%)	1/11 (9.09%)	0/11 (0%)	6/24 (25%)	0/6 (0%)	0/6 (0%)	5/44 (11.3%)	1/5 (20%)	0/5 (0%)
polycystic kidney	5/11 (45.4%)	0/5 (0%)	0/5 (0%)	3/6 (50%)	0/3 (0%)	0/3 (0%)	2/5 (40%)	0/2 (0%)	0/2 (0%)
isolated renal cyst	1/11 (9.09%)	1/1 (100%)	0/1 (0%)	0/6 (0%)	0/0 (0%)	0/0 (0%)	1/5 (20%)	1/1 (100%)	0/1 (0%)
multicystic kidney	5/11 (45.4%)	0/5 (0%)	0/5 (0%)	3/6 (50%)	0/3 (0%)	0/3 (0%)	2/5 (40%)	0/2 (0%)	0/2 (0%)
Urinary tract dilation	15/68 (22.0%)	0/15 (0%)	1/15 (6.66%)	4/24 (16.6%)	0/4 (0%)	1/4 (25%)	11/44 (25%)	0/11 (0%)	0/11 (0%)
UTD A1	8/15 (53.3%)	0/8 (0%)	1/8 (12.5%)	2/4 (50%)	0/2 (0%)	1/2 (50%)	6/11 (54.5%)	0/6 (0%)	0/6 (0%)
UTD A2-3	7/15 (46.6%)	0/7 (0%)	0/7 (0%)	2/4 (50%)	0/2 (0%)	0/2 (0%)	5/11 (45.4%)	0/5 (0%)	0/5 (0%)
Duplicated collecting system	2/68 (2.94%)	0/2 (0%)	0/2 (0%)	0/24 (0%)	0/0 (0%)	0/0 (0%)	2/44 (4.54%)	0/2 (0%)	0/2 (0%)
Duplicated collecting system	2/2 (100%)	0/2 (0%)	0/2 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)	2/2 (100%)	0/2 (0%)	0/2 (0%)
Renal hyper echogenicity	5/68 (7.35%)	1/5 (20%)	0/5 (0%)	2/24 (8.33%)	0/2 (0%)	0/2 (0%)	3/44 (6.81%)	1/3 (33.3%)	0/3 (0%)
hyperechogenicity	5/5 (100%)	1/5 (20%)	0/5 (0%)	2/2 (100%)	0/2 (0%)	0/2 (0%)	3/3 (100%)	1/3 (33.3%)	0/3 (0%)
Renal malformation	15/68 (22.0%)	1/15 (6.66%)	0/15 (0%)	4/24 (16.6%)	0/4 (0%)	0/4 (0%)	11/44 (25%)	1/11 (9.09%)	0/11 (0%)

	ALL(n=68)			ISOLATED(n=24)			ASSOCIATED(n=44)		
	N	Pathogenic	VUS	N	Pathogenic	VUS	N	Pathogenic	VUS
renal agenesis	6/15 (40%)	0/6 (0%)	0/6 (0%)	3/4 (75%)	0/3 (0%)	0/3 (0%)	3/11 (27.2%)	0/3 (0%)	0/3 (0%)
renal dysplasia	5/15 (33.3%)	1/5 (20%)	0/5 (0%)	0/4 (0%)	0/0 (0%)	0/0 (0%)	5/11 (45.4%)	1/5 (20%)	0/5 (0%)
renal hypoplasia	2/15 (13.3%)	0/2 (0%)	0/2 (0%)	0/4 (0%)	0/0 (0%)	0/0 (0%)	2/11 (18.1%)	0/2 (0%)	0/2 (0%)
complex genitourinary anomalies	4/15 (26.6%)	0/4 (0%)	0/4 (0%)	2/4 (50%)	0/2 (0%)	0/2 (0%)	2/11 (18.1%)	0/2 (0%)	0/2 (0%)
Bladder anomalies	7/68 (10.2%)	1/7 (14.2%)	0/7 (0%)	4/24 (16.6%)	1/4 (25%)	0/4 (0%)	3/44 (6.81%)	0/3 (0%)	0/3 (0%)
bladder exstrophy	1/7 (14.2%)	0/1 (0%)	0/1 (0%)	1/4 (25%)	0/1 (0%)	0/1 (0%)	0/3 (0%)	0/0 (0%)	0/0 (0%)
megacystis	4/7 (57.1%)	1/4 (25%)	0/4 (0%)	2/4 (50%)	1/2 (50%)	0/2 (0%)	2/3 (66.6%)	0/2 (0%)	0/2 (0%)
other bladder anomalies	2/7 (28.5%)	0/2 (0%)	0/2 (0%)	1/4 (25%)	0/1 (0%)	0/1 (0%)	1/3 (33.3%)	0/1 (0%)	0/1 (0%)

Table 18. Details of all cases with pathogenic CNVs in the different types of urinary anomalies.

Case	Gestational age (weeks)	cardiac anomaly	Other anomalies	ISCN formula	Genetic content	Comments	De novo inherited	Test	visible by karyotype	Follow-up
1	20	pelvic kidney	microretrognathia	4p16.3p15.33 (91545_14988069)x1	4p16.3 terminal deletion (Wolf-Hirschhorn syndrome)	Wolf-Hirschhorn syndrome	de novo	High resolution 8x60 oligonucleotide ISCA array)	YES	TOP
2	16.2	cervical lymphangiomas	ventriculomegaly,renal cyst, LCDH	Xq26.2(132315039_132876911)x0	Simpson-Golabi-Behmel syndrome type 1 (SGBS1; MIM:312870, ORPHA:373)	Ventriculomegaly, congenital diaphragmatic hernia, renal cyst: Simpson-Golabi-Behmel Syndrome Type 1. Genes, GPC4 (OMIM: 300168), GPC3 (OMIM: 300037)	maternal	High resolution 8x60 oligonucleotide ISCA array)	NO	TOP
3	13	megacystis	isolated	16p11.2(29673954_30332581)x3, 22q11.21(18894835_21809009)x1	Proximal 22q11.2 deletion (classic, LCR22A-LCR22D) (DiGeorge/velocardiofacial syndromes)	Síndrome de deleción 22q11.21 y síndrome de duplicación 16p11.2	de novo	High resolution 8x60 oligonucleotide ISCA array)	NO	TOP
4	26	renal dysplasia	complex cardiopathy	(14)x2~3	mosaic trisomy chromosome 2		de novo	High resolution 8x60 oligonucleotide ISCA array)	YES	TOP
5	14.1	hyperechogenic kidney	Hypoplastic Right Heart Syndrome, lip/palate bilateral cleft, hydrothorax, FGR	9p24.3p13.1(204090_38815471)x3~4	9p mosaic tetrasomy syndrome +i(9)(p24p12)	Clinical phenotype of tetrasomy 9p includes a variety of physical and developmental abnormalities. Commonly, patients have distinctive facial appearances with hypertelorism, cleft lip or palate, ear anomalies, and micrognathia. In addition, recurrent clinical features include developmental delay, central nervous system anomaly, limb defects, postnatal growth failure, congenital heart disease, renal anomalies, and short neck with excess nuchal skin	de novo	High resolution 8x60 oligonucleotide ISCA array)	YES	TOP
6	21	pelvic kidney	microretrognathia, FGR,cryptorchidism	7q11.23(72766313_74133332)x1	7q11.23 frequent deletion (95%) (Williams-Beuren syndrome, WBS)		de novo	High resolution 8x60 oligonucleotide ISCA array)	NO	TOP

6.8 STUDY 7: GENITAL ANOMALIES

Genital anomalies in which an array study had been performed, were found in 28 cases. One case of trisomy 13 diagnosed by QF-PCR was excluded. Finally, 27 cases were included in the study.

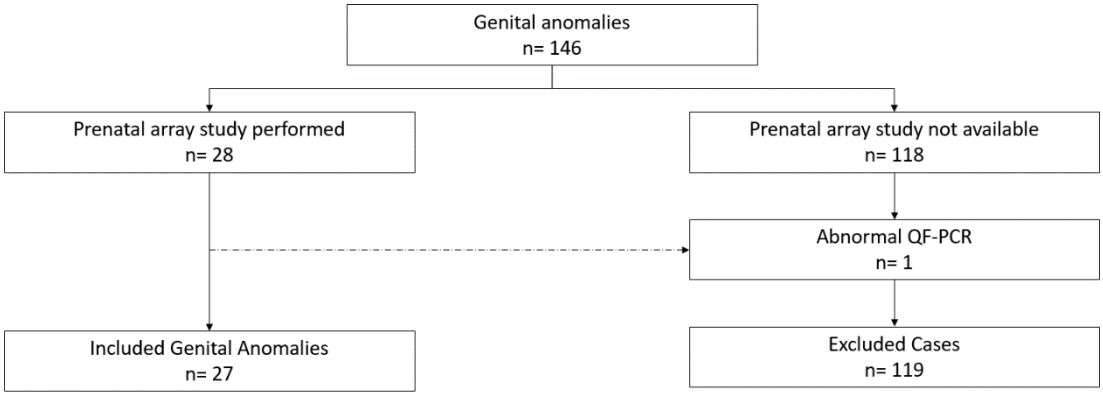


Figure 12: Genital anomalies study. Flowchart: selection criteria

Demographic characteristics

The median maternal age was 32 years (IQR, 17 to 43); the median gestational age at invasive testing was 23.5 weeks (IQR, 13.3 to 34). Fifteen women (55.6 %) were nulliparous, and 12 (44.4%) were multiparous. Twenty-two (81.5%) were singleton pregnancies, 4 (14.8 %) were dichorionic twin pregnancies, and 1 case (3.7%) was a monochorionic twin pregnancy.

Type of anomaly

Genital anomalies were found isolated in 2 (7.4%) cases; in all the other cases (n= 25, 92.6%), abnormal genitalia was associated with other major anomalies. Fetal growth

restriction was identified in 16 (59.3) out of the 27 cases. All the cases in which a fetal growth restriction was present (n= 16) belonged to the subgroup of abnormal genitalia and other extragenital anomalies. No cases of FGR were found in the subgroup of isolated genital anomalies.

Anomalies detected included ambiguous genitalia (n=4, 14.8%), penile anomalies (n=16, 59.3%) and testicular anomalies (n=7, 25.9%).

In the isolated subgroup (n=2), the anomalies identified were 2 penile anomalies.

In the associated subgroup (n=25), the genital anomalies diagnosed were: 4 cases (16%) of ambiguous genitalia, 14 cases (56%) of penile anomalies and 7 cases (28%) of testicular anomalies.

Array study

Regarding the type of invasive test, in cases 25 (92.6%), an amniocentesis was performed, in 1 (3.7 %) case chorionic villus sampling was performed, and in 1 case (3.7%), fetal tissue after a termination of pregnancy was obtained.

A pathogenic CNV was found in 4 out of the 27 cases (14.8%), a VUS in 1 case (3.7 %), and a normal result in the remaining 22 cases (81.5 %) of abnormal genitalia.

Table 19 shows pathogenic CNVs and VUS results in genital anomalies, either isolated or associated with other extragenital abnormalities.

No pathogenic CNVs were found in the two cases of isolated genital anomalies, while 4 cases of pathogenic CNVs (16 %) were diagnosed with associated anomalies. Similarly,

no cases of VUS were encountered in the isolated anomalies group compared to 1 case of VUS (4.0%) in the group of associated anomalies.

In 16 (59.3%) cases, a fetal growth restriction was diagnosed apart from the genital malformations, and all the FGR cases belonged to the associated anomalies group. Array results in genital anomalies with extragenital abnormalities and associated FGR were the following: 12 cases of normal array results and 4 cases (25%) of pathogenic CNVs; no variants of unknown significance were found in those cases presenting an FGR.

Table 20 reports the description of each case with pathogenic CNV

Table 19. Prevalence of pathogenic copy number variants (CNVs) and variants of unknown significance (VUS) in the different types of genital anomalies.

	ALL(n=27)			ISOLATED(n=2)			ASSOCIATED(n=25)		
	N	Pathogenic	VUS	N	Pathogenic	VUS	N	Pathogenic	VUS
Abnormal genitalia	4/27 (14.8%)	1/4 (25%)	0/4 (0%)	0/2 (0%)	0/0 (0%)	0/0 (0%)	4/25 (16%)	1/4 (25%)	0/4 (0%)
ambiguous genitalia	3/4 (75%)	1/3 (33.3%)	0/3 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)	3/4 (75%)	1/3 (33.33%)	0/3 (0%)
other genital anomalies	1/4 (25%)	0/1 (0%)	0/1 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)	1/4 (25%)	0/1 (0%)	0/1 (0%)
Penile anomalies	16/27 (59.2%)	0/16 (0%)	1/16 (6.25%)	2/2 (100%)	0/2 (0%)	0/2 (0%)	14/25 (56%)	0/14 (0%)	1/14 (7.14%)
hypospadias	14/16 (87.5%)	0/14 (0%)	0/14 (0%)	1/2 (50%)	0/1 (0%)	0/1 (0%)	13/14 (92.8%)	0/13 (0%)	0/13 (0%)
micropenis	2/16 (12.5%)	0/2 (0%)	1/2 (50%)	1/2 (50%)	0/1 (0%)	0/1 (0%)	1/14 (7.14%)	0/1 (0%)	1/1 (100%)
Testicular anomalies	7/27 (25.9%)	3/7 (42.8%)	0/7 (0%)	0/2 (0%)	0/0 (0%)	0/0 (0%)	7/25 (28%)	3/7 (42.8%)	0/7 (0%)
cryptorchidism	7/27 (25.9%)	3/7 (42.8%)	0/7 (0%)	0/2 (0%)	0/0 (0%)	0/0 (0%)	7/25 (28%)	3/7 (42.8%)	0/7 (0%)

Table 20. Details of all cases with pathogenic CNVs in the different types of genital anomalies.

Case	Gestational age (weeks)	cardiac anomaly	Other anomalies	ISCN formula	Genetic content	Comments	De novo inherited	Test	visible by karyotype	Follow-up
1	25	cryptorchidism	FGR	10q24.32(103077566_103453181)x3	10q24 continuous duplication (SHFM3) associated to Split-hand/Split-foot malformation 3, SHFM3, (OMIM 246560)	RefSeq (BTRC, POLL, RP11-529I10.4 i FBXW4) corresponds to Split-hand/Split-foot malformation 3, SHFM3,(OMIM 246560)	paternal	High resolution 8x60 oligonucleotide ISCA array)	NO	newborn: bilateral inguinal hernia, ventricular hypertrofia.
2	20	hypospadias	PLSVC, Single umbilical artery	8p23.1(11552465_11650630)x3	GATA 4			High resolution 8x60 oligonucleotide ISCA array)	NO	newborn: hypospadias, PLSVC.
3	34	cryptorchidism	FGR, polyhydramnios	15q11.2q13.1(22648239_28691601)x1 alt 22826753_27183335	Prader Willi/Angeman syndrome (BP1-BP3)	Prader-Willi syndrome	not studied		NO	TOP
4	21	ambiguous genitalia	FGR	15q11.2(22648239_23217655)x1	Deleción 15q11.2 (NIPA1)		maternal		karyotype: 47XYY	TOP
5	34	cryptorchidism	FGR, polyhydramnios	15q11.2q13.1(22648239_28691601)x1 alt 22826753_27183335	Prader Willi/Angeman syndrome (BP1-BP3)	Prader-Willi syndrome	not studied	High resolution 8x60 oligonucleotide ISCA array)	NO	TOP

6.9 STUDY 8: SKELETAL ANOMALIES

Fifty cases with skeletal anomalies in which an array-CGH study was performed were identified. From these, two cases were diagnosed with a fetal infection and therefore were excluded for the final analysis (1 case of Human Herpes virus and 1 case of Zika virus). Forty-eight cases were included in the final analysis.

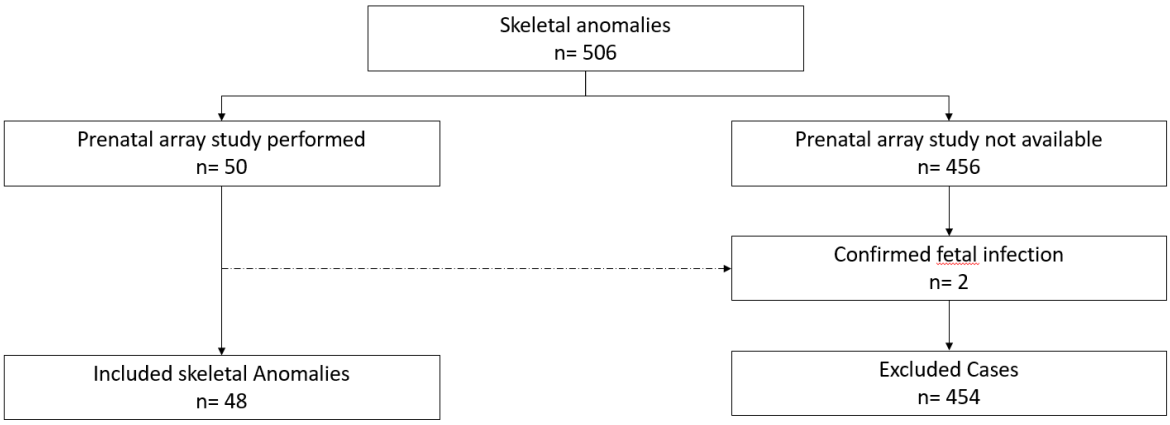


Figure 13: Skeletal anomalies study. Flowchart: selection criteria

Demographic characteristics

In the skeletal anomalies group, the median maternal age was 31 years (IQR, 16 to 46). At invasive test, the median gestational age was 21.6 weeks (IQR, 12.6 to 35). Twenty-seven (56.2%) women were nulliparous, and 21 (43.8%) were multiparous. Regarding the type of pregnancy, 44 (91.7%) were singleton pregnancies, and 4 (8.3%) were dichorionic twin pregnancies. No cases of monochorionic twin pregnancies or triples were found.

Type of anomaly

The skeletal anomaly was diagnosed as isolated in 37 (77.1%) out of the 48 cases; conversely, the abnormality was present with one more associated anomaly in 11 (22.9%) of the total cases.

An FGR was diagnosed in 6 cases (12.5%). All these cases were found in the subgroup with extra skeletal associated anomalies.

The skeletal anomalies identified were classified into groups as follows: spine anomalies (n=4, 8.7%), digital anomalies (n= 12, 26.1%), skeletal dysplasias (n=10, 20.8%) foot anomalies (n=18, 39.1%), and other skeletal anomalies (n=4, 8.7%).

If only isolated skeletal anomalies were considered, the groups of anomalies found were: spine anomalies (n=3, 8.1%), digital anomalies (n=9, 24.3%), skeletal dysplasias (n=9, 24.3%) foot anomalies (n=12, 32.4%), and other skeletal anomalies (n=4, 10.8%).

In case of skeletal defects associated with extra-skeletal anomalies (including the 6 cases of fetal growth restriction), the anomalies were represented as follows: spine anomalies (n=1, 9.1%), digital anomalies (n=3, 27.3 %), skeletal dysplasias (n=1, 9.1%) and foot anomalies (n=6, 54.5 %); no cases of other skeletal anomalies were found.

Array study

In 44 cases (91.7%), the invasive test performed was an amniocentesis; in 3 (6.2%) cases a chorionic villous sampling was performed, and in 1 case (2.1 %), a fetal sample (biopsy) for chromosomal analysis was obtained following a termination of pregnancy.

Considering the results of the array, a pathogenic CNV was diagnosed in 7 cases (14.6%), a VUS in 3 cases (6.2%), and a normal result was obtained in the remaining 38 (79.2%) cases.

In Table 21 details are given about all the pathogenic CNVs and VUS findings for the different skeletal anomalies, both for isolated and those with more associated anomalies.

If isolated skeletal anomalies were considered, in 4 of the 37 (10.8%) cases, a pathogenic CNV was diagnosed; conversely, in 3 of the 11 (27.3%) cases of skeletal anomalies with one or more associated major anomaly and associated FGR, a pathogenic CNV was diagnosed.

A VUS was found in 1 of the 37 (2.7%) cases with an isolated anomaly, and in 2 (18.2%) of the 11 cases with associated major anomalies and FGR.

Table 22 reports the description of each case with pathogenic CNV

Table 21. Prevalence of pathogenic copy number variants (CNVs) and variants of unknown significance (VUS) in the different types of skeletal anomalies.

	ALL(n=48)			ISOLATED(n=37)			ASSOCIATED(n=11)		
	N	Pathogenic	VUS	N	Pathogenic	VUS	N	Pathogenic	VUS
DIGITAL ANOMALIES	12/48 (25%)	3/12 (25%)	0/12 (0%)	9/37 (24.3%)	1/9 (11.1%)	0/9 (0%)	3/11 (27.2%)	2/3 (66.6%)	0/3 (0%)
oligosyndactyly	8/12 (66.6%)	3/8 (37.5%)	0/8 (0%)	6/9 (66.6%)	1/6 (16.6%)	0/6 (0%)	2/3 (66.6%)	2/2 (100%)	0/2 (0%)
polydactyly	4/12 (33.3%)	0/4 (0%)	0/4 (0%)	3/9 (33.3%)	0/3 (0%)	0/3 (0%)	1/3 (33.3%)	0/1 (0%)	0/1 (0%)
SKELETAL ANOMALIES	4/48 (8.33%)	0/4 (0%)	0/4 (0%)	4/37 (10.8%)	0/4 (0%)	0/4 (0%)	0/11 (0%)	0/0 (0%)	0/0 (0%)
reductional anomalies	1/4 (25%)	0/1 (0%)	0/1 (0%)	1/4 (25%)	0/1 (0%)	0/1 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)
limb body wall complex	1/4 (25%)	0/1 (0%)	0/1 (0%)	1/4 (25%)	0/1 (0%)	0/1 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)
micromelia	1/4 (25%)	0/1 (0%)	0/1 (0%)	1/4 (25%)	0/1 (0%)	0/1 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)
focal femoral agenesis	1/4 (25%)	0/1 (0%)	0/1 (0%)	1/4 (25%)	0/1 (0%)	0/1 (0%)	0/0 (0%)	0/0 (0%)	0/0 (0%)
SPINE ANOMALIES	4/48 (8.33%)	0/4 (0%)	0/4 (0%)	3/37 (8.10%)	0/3 (0%)	0/3 (0%)	1/11 (9.09%)	0/1 (0%)	0/1 (0%)
other vertebral anomalies	3/4 (75%)	0/3 (0%)	0/3 (0%)	2/3 (66.6%)	0/2 (0%)	0/2 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
hemivertebra	1/4 (25%)	0/1 (0%)	0/1 (0%)	1/3 (33.3%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/0 (0%)	0/0 (0%)
SKELETAL DYSPLASIA	10/48 (20.8%)	2/10 (20%)	0/10 (0%)	9/37 (24.3%)	2/9 (22.2%)	0/9 (0%)	1/11 (9.09%)	0/1 (0%)	0/1 (0%)
skeletal dysplasias	4/10 (40%)	2/4 (50%)	0/4 (0%)	4/9 (44.4%)	2/4 (50%)	0/4 (0%)	0/1 (0%)	0/0 (0%)	0/0 (0%)
hypochondroplasia	1/10 (10%)	0/1 (0%)	0/1 (0%)	1/9 (11.1%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/0 (0%)	0/0 (0%)
osteochondrodysplasia	1/10 (10%)	0/1 (0%)	0/1 (0%)	0/9 (0%)	0/0 (0%)	0/0 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)

	ALL(n=48)			ISOLATED(n=37)			ASSOCIATED(n=11)		
	N	Pathogenic	VUS	N	Pathogenic	VUS	N	Pathogenic	VUS
achondroplasia	2/10 (20%)	0/2 (0%)	2/2 (100%)	0/9 (0%)	0/0 (0%)	0/0 (0%)	0/1 (0%)	0/0 (0%)	0/0 (0%)
large bones below 5th centile	2/10 (20%)	0/2 (0%)	2/2 (100%)	0/9 (0%)	0/0 (0%)	0/0 (0%)	0/1 (0%)	0/0 (0%)	0/0 (0%)
FOOT ANOMALIES	18/48 (37.5%)	2/18 (11.1%)	3/18 (16.6%)	12/37 (32.4%)	1/12 (8.33%)	1/12 (8.33%)	6/11 (54.5%)	1/6 (16.6%)	2/6 (33.3%)
up/low extremities anomalies	1/18 (5.55%)	0/1 (0%)	1/1 (100%)	10/12 (83.3%)	1/10 (10%)	1/10 (10%)	1/6 (16.6%)	0/1 (0%)	1/1 (100%)
talipes equinovarus	15/18 (83.3%)	2/15 (13.3%)	2/15 (13.3%)	0/12 (0%)	0/0 (0%)	0/0 (0%)	5/6 (83.3%)	1/5 (20%)	1/5 (20%)
minor foot anomalies	2/18 (11.1%)	0/2 (0%)	0/2 (0%)	2/12 (16.6%)	0/2 (0%)	0/2 (0%)	0/6 (0%)	0/0 (0%)	0/0 (0%)

Table 22. Details of all cases with pathogenic CNVs in the different types of skeletal anomalies.

Case	Gestational age (weeks)	cardiac anomaly	Other anomalies	ISCN formula	Genetic content	Comments	De novo inherited	Test	visible by karyotype	Follow-up
1	21	clubfoot	isolated	16p12.2(21631626_21728426)x0	Deafness, autosomal recessive 22 (MIM: 607039)	OTOA	not studied	High resolution 8x60 oligonucleotide ISCA array)	NO	no follow up
2	23.2	oligosyndactyly	holoproseceph aly, fetal growth restriction	13q31.3q34(91571035_115093115)x1	deletion includes ZIC2 (MIM603073)		de novo	High resolution 8x60 oligonucleotide ISCA array)	YES	TOP
3	22	oligosyndactyly		10q21.1q21.2 (57068694_62787002)x1	10q21.1q21.2 deletion		de novo	High resolution 8x60 oligonucleotide ISCA array)	NO	TOP
4	13.1	skeletal dysplasia		Xp22.31(6628264_8050650)x0	Incidental pathogenic finding: Ichthyosis, X-linked (MIM:308100)		maternal	High resolution 8x60 oligonucleotide ISCA array)	NO	TOP
5	30	skeletal dysplasia		10q26.3q23.3 (93490443_125339893)x1	Huge deletion. Several critical regions of 10q26 deletion syndrome		de novo	High resolution 8x60 oligonucleotide ISCA array)	NO	TOP
6	25.5	oligosyndactyly	holoproseceph aly, fetal growth restriction, VSD	13q31.3 _ q34(92587094_115093115)x1	Deletion includes ZIC2 (MIM603073) associated with incomplete penetrance to holoprosencephaly 5 (MIM:609637)		de novo	High resolution 8x60 oligonucleotide ISCA array)	YES	TOP
7	21	clubfoot		4p16.3(75647_3776839)x1	Wolf-Hirschhorn syndrome	Wolf-Hirschhorn syndrome	de novo	High resolution 8x60 oligonucleotide ISCA array)	YES	TOP

6.10 STUDY 9: HYDROPS FETALIS

Twenty-five cases of hydrops fetalis in which an array-CGH study was performed were identified. From these, two cases were excluded: one case was diagnosed with a fetal infection (1 case of Human Herpes virus), and one case presented an abnormal QF-PCR (one case of trisomy 18). Twenty-three cases were therefore included in the final analysis.

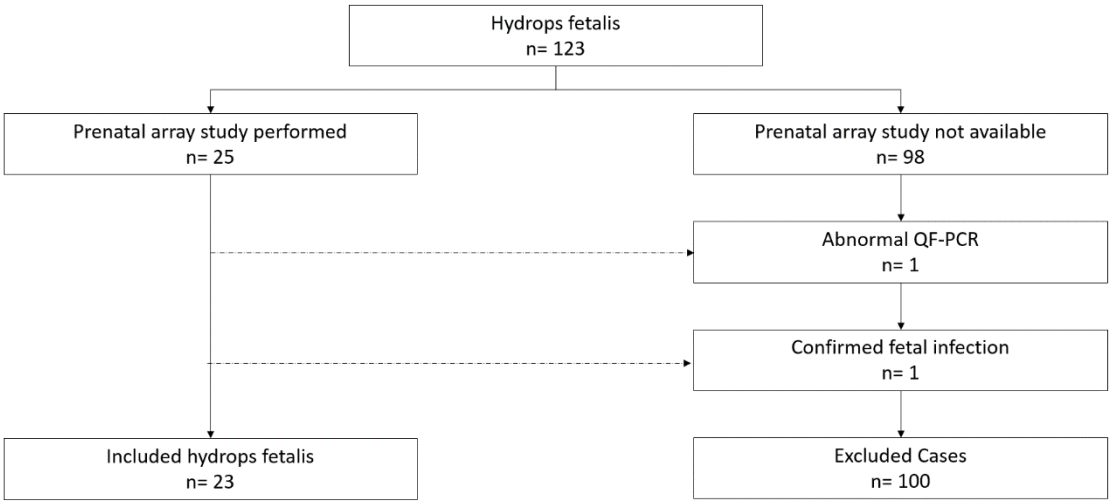


Figure 14: Hydrops fetalis study. Flowchart: selection criteria

Demographic characteristics

The median maternal age was 31 years (IQR, 19 to 35). At invasive test, the median gestational age was 18 weeks (IQR, 12.0 to 32.1); nine (39.1%) women were nulliparous, and 14 (60.9%) women were multiparous. All 23 cases (100%) were singleton pregnancies.

Type of anomaly

The cases of hydrops were present as isolated in 12 (52.2%) out of the 23 cases; conversely, the abnormality was present with one more associated anomaly in 11 (47.8%) cases.

Fetal growth restriction was diagnosed in one case (4.3%) and was found in the subgroup with associated anomalies; the remaining 22 (95.7%) cases did not present fetal growth restriction.

Among the 11 cases of hydrops with other associated anomalies, systems with anomalies included central nervous system anomalies (n=2, 18.2%), congenital heart defects (n=4, 36.4%); facial dysmorphisms (n=2, 18.2%); thoracic anomalies (n=3, 27.3%); gastrointestinal or abdominal wall anomalies (n=2, 18.2 %); renal anomalies (n=1, 9.1%); skeletal anomalies (n=2, 18.2%); Minor ultrasound anomalies were found in 3 cases (27.3%).

Array study

Amniocentesis was performed in 17 cases (73.9%), and in 6 (2.6%) cases, a chorionic villous sampling.

Considering the results of the array, a pathogenic CNV was diagnosed in 2 cases (8.7%), a VUS in another 2 (8.7 %), and a normal result in the remaining 19 (82.6%) cases.

In Table 23 details are given about all the pathogenic CNVs and VUS findings for the in case of hydrops both for isolated and for those cases of hydrops with more associated anomalies.

If isolated hydrops are considered, in 1 of the 12 (8.3%) cases, a pathogenic CNV was diagnosed; in 1 of the 11 (9.1%) cases of hydrops with one or more associated major anomaly or associated FGR, a pathogenic CNV was diagnosed.

No VUS were found in the case of isolated hydrops; conversely, a VUS was detected in 2 (18.2%) of the 11 cases with associated major anomalies and FGR.

Table 24 reports the description of each case with pathogenic CNV

Table 23. Prevalence of pathogenic copy number variants (CNVs) and variants of unknown significance (VUS) in the different types of hydrops anomalies.

	ALL(n=23)			ISOLATED(n=12)			ASSOCIATED(n=11)		
	N	Pathogenic	VUS	N	Pathogenic	VUS	N	Pathogenic	VUS
Hidrops	23/23 (100%)	2/23 (8.69%)	2/23 (8.69%)	12/12 (100%)	1/12 (8.33%)	0/12 (0%)	11/11 (100%)	1/11 (9.09%)	2/11 (18.1%)
FGR	1/23 (4.34%)	1/1 (100%)	0/1 (0%)	0/12 (0%)	0/0 (0%)	0/0 (0%)	1/11 (9.09%)	1/1 (100%)	0/1 (0%)
No FGR	22/23 (95.6%)	1/22 (4.54%)	2/22 (9.09%)	12/12 (100%)	1/12 (8.33%)	0/12 (0%)	10/11 (90.9%)	0/10 (0%)	2/10 (20%)

Table 24. Details of all cases with pathogenic CNVs in the different types of hydrops anomalies.

Case	Gestational age (weeks)	cardiac anomaly	Other anomalies	ISCN formula	Genetic content	Comments	De novo inherited	Test	visible by karyotype	Follow-up
1	12.1	hydrops	isolated	3q24q29(143375759_197837069)x3	Very large duplication that includes 3q29 duplication syndrome. Chromosome 21 region has not relevant genetic material and is not covered by arrays	mother's karyotype 46,XX,t(3,21)(q23:p11.2)	de novo	High resolution 8x60 oligonucleotide ISCA array	NO	TOP
2	14.1	hydrops	Hypoplastic Right Heart Syndrome,bilateral lip/paladar cleft; ; fetal growth restriction; hyperechogenic kidney	9p24.3p13.1(204090_38815471)x3~4	9p mosaic tetrasomy syndrome +i(9)(p24p12)	Clinical phenotype of tetrasomy 9p includes a variety of physical and developmental abnormalities. Commonly, patients have distinctive facial appearances with hypertelorism, cleft lip or palate, ear anomalies, and micrognathia. In addition, recurrent clinical features include developmental delay, central nervous system anomaly, limb defects, postnatal growth failure, congenital heart disease, renal anomalies, and short neck with excess nuchal skin.	de novo	High resolution 8x60 oligonucleotide ISCA array)	YES	TOP

PART 7

DISCUSSION

7 DISCUSSION

7.1 OVERALL

The prevalence of pathogenic CNVs and VUS was investigated in a cohort of 648 fetuses with different major malformations and/or fetal hydrops. Systems included were: Central Nervous System, cardiac, thoracic, gastrointestinal, urinary, facial, genital, and skeletal with or without associated fetal growth restriction or hydrops.

In our cohort we found an overall prevalence of pathogenic CNVs in 8.3% of cases and variants of unknown significance in 4.3 % of the total.

The prevalence of pathogenic CNVs varied between different groups of malformations. The lowest prevalence was found in case in case of gastrointestinal anomalies with 5.6% of pathogenic CNVs; the highest prevalence was found in case of genital anomalies with a 14.8 % of pathogenic CNVs.

The prevalence of variants of unknown significance varied from the lowest in case of urologic anomalies, with 1.5% of VUS detected, to the highest in case of central nervous system anomalies and hydrops with an 8% and 8.7% respectively of VUS.

If only isolated cases are considered, the highest prevalence of pathogenic results was found in case of cardiac anomalies with 10.4% of pathogenic CNV, and in skeletal anomalies with 10.8% of pathogenic results. The prevalence of VUS in isolated cases varied from the lowest in case of facial anomalies, genital anomalies, and fetal hydrops with no variants of unknown significance detected, to the highest in case of central nervous system anomalies with 7.7% of VUS detected.

We confirmed that microarray a robust method to detect more clinically significant chromosomal imbalances in fetuses with ultrasound anomalies and it is a technique that should be used as a first-tier genetic test together with a rapid test (QF-PCR) in prenatal diagnosis.

7.2 CNS ANOMALIES

Main findings

We investigated the prevalence of pathogenic CNVs and VUS detected by CGH-Array in a cohort of 238 fetuses with different CNS anomalies. Our results showed a similar prevalence of 7% for CNVs and 8% of VUS once aneuploidies of chromosomes 13, 18, 21, X and Y were excluded.

The prevalence of pathogenic CNVs was higher when other structural abnormalities were present (18.4% of cases with an associated anomaly versus 3.8% of cases of isolated anomalies). On the other hand, VUS showed similar results (8.2% if associated anomalies were present versus 7.7 % if the detected anomaly was isolated), reflecting the probable lack of relevant phenotypic effect of most of the VUS and adding evidence in favor of the policy of not reporting them prenatally.[196,197] Interestingly, when isolated anomalies involved either the posterior fossa or cortical development, a higher prevalence of pathogenic CNVs and VUS was observed. In isolated CNS anomalies, the malformations with a higher prevalence of pathogenic CNVs were cerebellar hypoplasia (33%), mega cisterna magna (20%), moderate ventriculomegaly (11%) and spina bifida (3.7%).

Results in the context of what is known

The use of CMA in the prenatal setting has proven to be an excellent tool compared to standard cytogenetic karyotyping in the diagnosis of chromosomal anomalies in the case of fetuses carrying one or more major malformations and a normal karyotype.[194,224.]Several studies have reported the prevalence of pathogenic CNVs and VUS in fetuses with ultrasound anomalies. The prevalence of pathogenic CNVs in these cases is about 5-7%.[194,198,225,226] However, fewer studies have explicitly reported on specific CNS anomalies,[227–230] the prevalence of pathogenic CNVs in these studies in fetuses with a normal karyotype varies between 3.7% and 10.9%. Our results show a similar prevalence of pathogenic CNVs, 4.2% (10 out of 238) if only CNVs below the resolution of karyotype analysis are considered.

We found 6 cases of pathogenic CNVs that would have been potentially visible also if a standard karyotype had been performed (abnormalities larger than 5-10 Mb).[231]

The highest prevalence of pathogenic CNVs in our study was found for posterior fossa anomalies (12.9%), being cerebellar hypoplasia the most prevalent (33%), followed by ventriculomegaly (8.4%). These data are consistent with the available literature.[232,233] Zou *et al*. report a prevalence of 10.8% in cases of posterior fossa anomalies, with the highest prevalence in those cases of cerebellar hypoplasia (25%), vermian hypoplasia and Dandy-Walker anomal.[234]

There are broad differences in the prevalence of VUS described in the literature for several reasons. First, and besides the type of array employed (CGH, SNPs BACs), different reporting policies exist in different countries and laboratories. Therefore, VUS

might be under-reported since most studies may have considered only patient informed VUS, but not all detected VUS. Second, as in pathogenic CNVs, there might be a selection bias in those studies performed on patients who underwent termination of pregnancy. Third, reported cohorts involving CNS anomalies usually involve smaller cohorts than our study, being more prone to random fluctuations. Our study has considered all detected CNVs, whether reported or not, in a cohort of 238 fetuses presenting CNS anomalies, and we found a higher prevalence than other studies. A prevalence of 4.8% has been reported in a more extensive general study involving 2858 pregnancies with ultrasound anomalies[198] and of 5.2% in a metanalysis involving 799 fetuses with ultrasound anomalies.[194] Lower diagnostic yield of both studies can be easily explained by the inclusion of a significant proportion of BAC-based array CGH analysis, a low-resolution technology currently replaced by the oligonucleotide-based technology used in our study. Considering literature data focusing on CNS anomalies, our results are comparable to those of Sun *et al.* reporting a prevalence of VUS in CNS anomalies of 6.5% in 46 subjects,[227] but lower than the prevalence reported by Schumann *et al*. with a 27% of VUS in 33 fetal samples derived from termination of pregnancy.[228] However, the high frequency in the later study could reflect fluctuations due to the relatively low number of fetuses included.

Some recommendations about the use of microarray in the prenatal setting suggest not to inform about alterations that are not clearly associated with pathogenic phenotypes in current literature.[196,197] Not all the groups working in the prenatal field agree with this clinical conduct since this could be considered a paternalistic vision in an era where the self-determination of patients is arising; this is especially true in the case of a fetus

whose legal definition changes across different countries. On the other way to report prenatally, all the variants could generate anxiety in the parents and physicians in those cases in which it has to be decided about the future of an unborn child, in a moment in which it is not possible to confirm exactly the phenotype.[233] Regarding the specific involvement of genes, *SOX3* duplication has been recently associated with neural tube defects.[211,212] In our series, we found *SOX3* duplication in a male fetus affected with spina bifida.

We also detected a de novo 1p36 deletion in a fetus showing abnormal cerebral sulcation associated with cleft lip. A recent series of cases with 1p36 deletion suggests association to brain and facial abnormalities and reinforcing the indication of CMA study when these prenatal findings are detected. The authors postulate that 1p36 deletion is difficult to diagnose given that it can be present without specific ultrasound signs.[235]

Limitations of the study

Our study is retrospective and based only on pregnancies that underwent an invasive test. It is likely that some women declined an invasive test in cases of mild or CNS anomalies, and we don't have the postnatal follow up of those children.

Clinical implications

Our study provides further evidence to recommend the use of MCA studies that, in our opinion, should be the first-tier test over karyotype, multiple ligation-dependent probe amplification and fluorescent in situ hybridization techniques for prenatal diagnosis.[194,236]

The large sample size analyzed allowed us to perform a sub analysis for each type of anomaly, providing more detailed information regarding the prevalence of pathogenic CNVs and VUS in specific brain anomalies detected prenatally.

In our opinion, this study could help to better define the prognosis of CNS anomalies, especially those considered isolated in prenatal diagnosis and could help to reclassify in the future some VUS through the association between fetal anomalies and the arising evidence about genetic alterations.

We stress the importance of a collaborative basis between the Obstetrics and Genetics Departments for direct access to the complete CMA data analysis allowing us to find in our study a higher prevalence of VUS, frequently omitted in final reports of CMA results in the prenatal setting.[237]

7.3 CARDIAC ANOMALIES

Main findings

For the sub-analysis of cardiac anomalies, we counted with a cohort of 191 fetuses with different cardiac abnormalities. Our group studied the prevalence of different CNVs (pathogenic CNVs and VUS) detected with CMA after excluding common aneuploidies (Trisomy 21, 18, and 13). We found an overall prevalence of 13.1% for pathogenic CNVs and 3.7% for VUS. If other anomalies, a part of the cardiac ones, were present, the prevalence of pathogenic CNVs was higher: 18.2% in the case of associated anomalies versus 10.4 % in isolated cardiac alterations. Also, in the case of VUS, the prevalence was different in both subgroups (7.6% if associated anomalies versus 1.6 % if isolated anomalies).

In isolated cardiac defects, left and right heart malformations (16.7%), conotruncal malformations (14.6%), and septal defects (12.5%) were the groups of anomalies that presented a higher prevalence of pathogenic CNVs. On the other hand, left heart disease (5.6%), and other cardiac anomalies (cardiomyopathies) (11.1%) presented a higher prevalence of VUS.

In the isolated subgroup, the single cardiac anomalies with the overall highest prevalence of pathogenic copy number variants were pulmonary atresia (66.7%), Tetralogy of Fallot (30.8%), aortic arch anomalies (interrupted arch, and right aortic arch: (14.3%)), and ventricular septal defects (16.7%).

Results in the context of what is known

The prevalence of CNVs of clinical significance in prenatal cases of cardiac anomalies is described in few cohorts, and the majority of reports that have been published in recent years focused on CHDs in general, without performing analyses of specific defects.[88,198] A meta-analysis detected a prevalence of pathogenic CNVs in 7% and an additional 3.4% for VUS of fetuses with a cardiac anomaly and normal karyotype. [85] A study performed by Schmid *et al.* [238] found potential causal CNVs in 25% of fetuses with a cardiac anomaly with normal karyotype and negative FISH. Yan *et al.* reported an incremental yield of microarray over karyotype in 6% of isolated CDH and in 7.4% of CDH with more associated anomalies. [88]

Our results show an overall higher prevalence of pathogenic CNVs, 13.1% (25 out of 191). After excluding cases with 22q11 deletion, the prevalence of pathogenic CNVs was 9.9%. Even if only isolated anomalies were considered, however, our results show a

higher prevalence to the one of literature: we found a 10.4 % of pathogenic CNVs (including DiGeorge/velocardiofacial Syndrome) and 5.6% if cases with 22q11 deletion were excluded in case of isolated cardiac alterations compared with a 3.4% incremental yield over karyotype in the study of Jansen *et al*. [85] and compared with the one Mademont-Soler reported (2% of incremental yield once 22q11.2 microdeletions were excluded). [68] As a matter of facts we need to highlight that our hospital is a referral centre for the study of prenatal and postnatal cardiopathies. We do think that this may explain the reason why the overall prevalence of pathogenic variants is higher. The centre acts as a funnel for more severe and complicated cases and the number of invasive test and array studies performed is consequently higher.

Array CGH presents an advantage over standard techniques regarding the analysis for 22q11 microdeletions (FISH analysis) since it can detect atypical deletions not visible with standard FISH. [239]

If postnatal results are considered, the prevalence of pathogenic CNVs reported is higher and varies between 4% [240] up to 25% [241] and 27.9% in the study of Wu at al [242]. On one hand, different arrays setups were used in these studies, and pathogenic CNVs detection rates ranged consistently (as a general rule, arrays with high resolution will lead to a proportional increase in the number of pathogenic CNVs).[242,243] On the other hand, the higher prevalence in postnatal reports probably reflects the general limitation of prenatal ultrasound in detecting dysmorphic alterations and subtle expressions of syndromic anomalies.

The highest prevalence of pathogenic alterations for isolated CHD was found in left heart defects (pathogenic CNVs 16.7%), conotruncal malformations (pathogenic CNVs 14.6%),

and septal defects (pathogenic CNVs 12.5%); in literature heterogeneous results are reported. [85,198] In a study of Lin *et al*., a prevalence of 5.3% of pathogenic CNVs is reported in cases of conotruncal anomalies (including 22q11.21 microdeletions), and they found a higher prevalence of pathogenic variants in cases on conotruncal heart defects associated with other extracardiac anomalies; [244] when we considered this subgroup of anomalies we found a higher prevalence of pathogenic CNVs in the isolated group than in the associated one (14.6% vs 7.7%).

Considering left heart defects, we found an overall incremental yield of 16.7% in case of isolated anomalies; postnatally, it is reported that 5-12 % of cases are associated with chromosomal abnormalities (included monosomy X), 22q11.2 microdeletion syndrome and 11q deletion syndrome. [245] Shaffer *et al.*, reported an incremental yield of significative CNVs of 9.5% in a subgroup of isolated HLHS fetuses; [198] another report by Hitz *et al*. stated that up in 10% of left-sided defects, a CNVs is present with a causative or contributing role in the anomaly. [246] In this last report, however, the postnatal phenotype was analyzed to include only true isolated left heart defects, whereas known syndromes and dysmorphic features were excluded. For this reason, these data are not truly applicable for prenatal counselling to parents since this information is lacking in the prenatal setting.

In our sub-analysis, transpositions of great arteries without associated anomalies, traditionally considered to present a low association with chromosomal abnormalities,[85] were associated with pathogenic CNVs in 5.6% of cases.

In our series, we found an unexpected high prevalence of pathogenic results in case of ventricular septal defects, with an overall prevalence of 16.6% (1 out of 6). In literature,

few reports are available regarding the prevalence of pathogenic CNVs in case of isolated VSD: VSD are described in 14% of cases of 22q11 deletion syndrome. The rate of other chromosomal anomalies is under debate, presenting a large variability due to demographics, race, year of data collection, and regional differences in prenatal reports, varying from 1.2 % in a study of Gomez *et al.* [247] (only aneuploidies were considered) to 3.4% in a study conducted by Liu *et al*..[248] Moreover, in our series, only one case of 6 had a pathogenic CNV. This finding shows two facts: firstly, most fetuses with VSDs did not undergo an invasive test, as we know that VSD is one of the most frequent cardiac defects (it is diagnosed in one-third of all heart defects diagnosed in the first year of postnatal life[249]), and an invasive test may have been performed only in those cases with large defects. Secondly, the confidence interval is wide, 3% to 56%, and therefore, the real prevalence could be any between these two limits.

Regarding VUS, as we mentioned in the sub-analysis of CNS, in literature data are not uniform for different reasons: different array platforms, reporting policies, biases derived from the type of cohort considered, and random fluctuations. Our finding of 3.7% of VUS in all cardiac anomalies is slightly lower than the data reported in the literature: Yan *et al*. reported a prevalence of 5.3% of VUS. [88]

Limitations of the study

The limitations are the same we reported for the CNS sub analysis; our study is retrospective and based only on pregnancies that underwent an invasive test. It is likely that some women declined an invasive test preventing having access to the real prevalence for all the different types of CHD; moreover, we don't have the postnatal follow up of most of those children. This is particularly true in frequent and mild

congenital cardiac defects, such as VSDs, where we found an unexpected high prevalence of pathogenic CNVs, which probably does not reflect the real prevalence as the vast majority of fetuses with VSD did not undergo an invasive test.

Clinical implications

In different studies, the classification of cardiac anomalies is not always consistent, which reduces the possibility of inferences of the CMA influence per specific cardiopathies; we were able to perform sub-analysis for subgroups of different types of cardiac anomalies allowing a more precise correlation between the type of CHD and the possible array anomalies and their prevalence depending on the specific type of cardiac anomaly. Some cardiac anomalies (such as great arteries transposition) were traditionally considered to have a low risk for genetic abnormalities, but even in these cases, we found a higher prevalence of pathogenic variants, emphasizing the importance of a complete genetic analysis, including microarray. Moreover, CMA can substitute FISH analysis and be performed as a first-tier test to detect 22q11.2 microdeletions.

7.4 THORACIC ANOMALIES

Main findings

We performed a sub-analysis in a cohort of 68 cases of fetuses with different kinds of thoracic anomalies after 3 cases (4.2%) in which the array study was run, but the genetic results were not available due to a technical failure were excluded. No cases with an abnormal QF-PCR were present. We encountered an overall prevalence of pathogenic variants in 10.3 % and VUS in 5.9% of the cases. When thoracic anomalies with

associated extra thoracic abnormalities (including fetal growth restriction) were considered, the prevalence of pathogenic CNVs was higher: 13.3% compared with 7.9% in cases of isolated thoracic anomalies.

If VUS were considered, the prevalence did not present a significant difference between the group of associated anomalies (with 6.7% of VUS) and the isolated group, where a prevalence of 5.3% was found.

In isolated thoracic anomalies, the highest prevalence of pathogenic variants was found in fetuses in the hydrothorax group (20% of pathogenic CNVs). The second most frequent was in the group of congenital diaphragmatic hernia (4.8% of pathogenic CNVs). None of the other's groups of isolated thoracic anomalies seemed associated with pathogenic variants.

The single isolated thorax anomalies with the highest prevalence of pathogenic CNVs were hydrothorax with a prevalence of 20.0% and left-sided congenital diaphragmatic hernia with a prevalence of 5.3%.

Results in the context of what is known

The etiology of some thoracic anomalies is still under debate, being some of them more than others associated with genetic anomalies. In recent years some authors published on thoracic anomalies and array, most of them did not include a sub-analysis for different kinds of abnormalities or focused only on a single thoracic anomaly.

Donnelly and colleagues reported a prevalence of pathogenic copy number variants of 15% in a cohort of 40 cases of thoracic anomalies and 4.6% (one case out of 22 isolated anomalies) if only isolated anomalies were considered. [63] Another study by Shaffer and

colleagues involving fetuses with ultrasound anomalies found 6.3% of pathogenic CNVs in a subgroup of 48 single system (respiratory) anomalies. [198] We found a higher prevalence of pathogenic results with an overall incremental yield of 10.3%, a possible reason can be found in the higher number of cases we were able to include in our study.

Hydrothorax was the single isolated anomaly in our series with the highest prevalence of pathogenic CNVs (20%) and VUS (10%). Hydrothorax is frequently present as a sign of non-Immune hydrops fetalis (NIHF): a wide variety of underlying genetic causes can lead to NIHF. It is reported that lymphatic vessel dysplasia and obstruction can cause NIHF in 5-6% of the cases.[185]

Fetal hydrothorax and cystic hygroma are common also in Turner syndrome;[250] we found a case presenting with hydrothorax and general subcutaneous edema with a normal QF-PCR that on the array studied revealed being a mosaic Turner.

The second single anomaly in which a higher prevalence of clinically significant results emerged in our study was left-sided CDH: we found an incremental yield of 5.3% of pathogenic CNVs in left-sided CDH (in the isolated group) and 20% in the associated group. In literature, CDH is reported to present more association with clinical significance variants than other thoracic anomalies; Donnelly *et al*. also reported on single thorax anomalies, and they found an incremental yield of 10% for CDH and 16.7% for cystic lung lesions.[63]

No pathogenic CNVs were found in cases of right-sided congenital diaphragmatic hernia. Right-sided CDH is reported with less frequency in literature, and our results reflect this incidence: right-sided CDH were 4 times less frequent than left-sided CDH. Furthermore,

no CNVs were found in our cohort (either pathogenic or VUS) making it difficult to drive inferences about the possible genetic contribution of CNVs detectable with CMA to right-sided CDH.

Among the 2 cases of left-sided CDH with pathogenic CNVs, we found a deletion in Xq26.2 (which include *GPC3* gene, associated with Simpson-Golabi-Behmel Syndrome). In the 2 cases, the progenitor's study evidenced the same genetic alteration in the phenotypically normal mother, as expected with a gene showing autosomal recessive X-linked inheritance. Although "De novo" mutations seem to constitute a significant fraction of the genetic alterations predisposing fetuses to develop CDH, for the cases found in our series, inheritance seems related to an X-linked transmission as reported in different reports in the literature.[135, 251,252] CDH is described in Xq26.2 deletions as a part of the spectrum that characterizes the genetic anomaly (OMIM 312870) with an association up to 10-20% of the cases.[251,252]

Limitations of the study

Our study is retrospective and based only on pregnancies that underwent an invasive test. Although we performed a sub-analysis for single thoracic anomalies, since the total number of some anomalies was limited, we could not count clinically significant CNVs or VUS in some sub-groups, and a significant conclusion can not be driven for some of the anomalies of the thoracic group.

Clinical implications

The prenatal characterization of the unique features associated with each case could be helpful for the diagnosis of more chromosomal abnormalities leading to specific fetal

structural anomalies. From this, prenatal counselling could be more precise and invasive testing could be offered in all cases of thoracic defects with a high suspect of genetic cause.

7.5 GASTROINTESTINAL ANOMALIES

Main findings

The group of gastrointestinal anomalies under study included 89 cases in which an array study had been performed. The prevalence of different CNVs (pathogenic CNVs and VUS) detected with CMA was evaluated after the exclusion of aneuploidies (Trisomy 21, 13,18 and 9), fetal infections with an intestinal ultrasound sign that could create a bias for the final analysis (cytomegalovirus and human herpes virus confirmed fetal infections), and failures in the microarray analysis with no results for the CMA.

We found an overall prevalence of 5.6% for pathogenic CNVs and 5.6% for VUS. The prevalence of pathogenic CNVs was higher in cases with associated anomalies (9.5% versus 2.1% in isolated cases). A variant of unknown significance was found less frequently in cases with an isolated gastrointestinal anomaly, with a prevalence of 4.3% versus 7.1% for cases with associated major anomalies and fetal growth restriction.

When isolated intestinal anomalies were considered, abdominal wall defects was the subgroup of anomalies with the highest prevalence of pathogenic CNVs (12.5%). Also, in the case of VUS, the highest prevalence was found in the group of abdominal wall abnormalities (12.5%), followed by intestinal anomalies (4.5%). None of the remaining groups of isolated gastrointestinal anomalies seemed associated with pathogenic variants.

The single isolated anomaly with the highest prevalence of pathogenic CNVs was omphalocele, with a prevalence of 20%.

Results in the context of what is known

Few studies have reported specifically on array study in case of gastrointestinal anomalies. Shaffer *et al*. reported an incremental yield of the array over karyotype of 11.1% in a group of gastrointestinal anomalies. [61] Donnelly *et al*. reported a prevalence of pathogenic CNVs of 10.8% in the case of non-isolated gastrointestinal anomalies but did not find any clinically significant variant in the case of isolated anomalies.[63] However, the total number considered was 9 fetuses for the study of Shaffer and 37 for the one of Donnelly and colleagues, and this can explain the higher prevalence reported if compared with the results of our group.

Omphalocele is the most common abdominal wall defect, with a higher frequency in prenatal reports than postnatal, mainly due to its high frequency of associated anomalies (approximately 40% of the cases) and aneuploidy (abnormal karyotype is found in 50% of the cases) that often drive to stillbirth or termination of pregnancy.[149,253] Microdeletions and duplications can play a significant role in those cases in which a common trisomy (usually trisomy 18 and 13) is discarded. However, there are few case reports of prenatal diagnosis of omphalocele and microarray; in a retrospective series focusing on omphalocele, the yield of pathogenic CNVs with CMA testing was 1.2% (1 case out of the total). [254] We found a similar incremental yield of 1.1% if the result was compared to the total cohort of gastrointestinal anomalies (n=89) (20% for both pathogenic CNVs and VUS if the result was compared against all the cases diagnosed

with omphalocele of the subgroup of all abdominal wall defects). In all cases the abdominal anomaly was present as isolated.

Regarding esophageal atresia, as for omphalocele, there are very few reports in the literature, and they present prenatal results comparable with our work: in a series by Rohanizadegan *et al.* it is reported a microarray study only in 17% cases (5 out of 29 cases) in a prenatal setting, and in 57% of the total cases in a second postnatal study. Although CMA was the most common postnatal test, none of the prenatal or postnatal CMAs led to a genetic significant diagnosis. In our cohort, we found an incremental yield of CMA in the case of esophageal atresia of 11%. The case of our sample presented a duplication in 14q11.2q21.3, derived from a balanced maternal translocation t (14;21)

In our cohort, we also report a case of fetal ascites in a fetus with other associated anomalies and a gain of DNA detected by CMA in the context of a tetrasomy 9 due to a short arm isochromosome 9, a chromosome anomaly that shows a strong propensity to tissue-limited mosaicism, since it occurs predominantly in peripheral blood cultures, often at a lower frequency or even absent in skin, amniotic fluid, or chorionic villous cell cultures. The tissue-limited nature of mosaicism may render prenatal detection of this condition very difficult.[255] In a prenatal cohort of fetal tetrasomy 9, an association is reported with fetal ascites and hydrops fetalis.[256]

Postnatal described cases show a milder phenotype characterized by ear malformations, skeletal and joint problems (especially dislocations), hypoplasia of digits and nails, cleft lip and palate, hypertelorism, urogenital abnormalities, bulbous/beaked nose, and congenital heart disease. [255]

Limitations of the study

Our study is retrospective in its nature. Although we performed a sub-analysis for single groups of anomalies, since the total number of some anomalies is limited, we did not find clinically significant CNVs or VUS in some sub-groups. For this reason, a conclusion can't be driven for some of the anomalies of the gastrointestinal group. Moreover, some soft markers, such as hyperechogenic bowel alone, could have escaped an invasive test and were not included in our study.

Pathogenic CNV and VUS were found in 4 cases each in the subgroup of associated anomalies. Although the results may indicate a relation between genetic alterations and gastrointestinal anomalies, being the absolute number small, and being all the cases not isolated, it is challenging to conclude whether these genetic alterations are connected directly to the above-mentioned gastrointestinal anomalies.

Clinical implications

Our data confirm the importance to perform an array study also in apparently isolated gastrointestinal anomalies in which common aneuploidies are discarded since it increments the diagnostic of genetic anomalies and permits a more precise prenatal counselling to families.

7.6 FACIAL ANOMALIES

Main findings

We investigated the prevalence of pathogenic CNVs and VUS detected by CGH-Array in a cohort of 66 fetuses with different facial anomalies.

Our results showed an overall prevalence of 16.7 % for pathogenic CNVs and 6.1% of VUS.

In the isolated group no variants of clinical significance were encountered; the prevalence of pathogenic CNVs when other structural abnormalities were present was 25%. Also, VUS showed similar results with a prevalence of 9.1% if associated anomalies were present and no cases of VUS if the detected anomaly was isolated.

In the non-isolated findings, the highest prevalence was found in mouth/lips anomalies, with a prevalence of 28.6%. Pathogenic findings were present in all the subgroups of non-isolated facial anomalies.

Results in the context of what is known

Shaffer *et al*. reported about fetuses with structural abnormalities: in fetuses with facial features, they found 4.3% of significant CNVs and 7.1% of VUS.[198] Donnelly and colleagues found an incremental yield of 15.2% (10 % if only isolated facial anomalies were considered). [63] We did not find any significant anomaly if facial abnormalities were present as an isolated finding. In the isolated subgroup, most of the anomalies found were lip/palate clefts, supporting the hypothesis that isolated facial clefts present a lower association with syndromes and chromosomal abnormalities. In the literature, a similar rate of clefts in both isolated and associated cases is reported when the diagnosis is made in the second trimester [257], but in our cohort, the frequency of clefts was higher in isolated anomalies than in non-isolated. One possible reason is that some severe cases detected in the first trimester had additional major anomalies and were terminated before an invasive test procedure, and therefore we are missing those data.

It is important to keep in mind that some anomalies associated with syndromes may not be visible in a prenatal setting: a study from the Netherlands found that 5% of a cohort of isolated clefts had an underlying genetic anomaly. [257] De Wit and colleagues found a 3-8% chance of finding a submicroscopic genetic alteration even if the ultrasound anomaly was apparently restricted to one system.[258] Also, Maarse *et al*. stress the importance that in facial clefts, even if the anomaly seems isolated, an array-CGH analysis should be offered to parents for a more accurate prenatal counselling and to help reduce the frequency of abnormal genetic findings diagnosed after birth. [259]

No clefts involving only the lips were found in our report; in literature, it is reported that the presence of isolated cleft lip seems less associated with genetic alteration and syndromes. It is possible that isolated clefts involving only the lip did not undergo an invasive test, and therefore were not included in our cohort.

On the other hand, in the group of lip and palate clefts, both pathogenic CNVs and VUS were present in our series in 28.6% and 14.3% of cases, respectively, confirming the higher association of the anomaly with genetic abnormalities in non-isolated cases.

In the non-isolated group, pathogenic CNVs were also found in case of eye anomalies (25%) (hypertelorism), nose anomalies (25%) (nasal bone agenesis), ear anomalies (25%) (implantation anomalies), and in profile anomalies (25%) reflecting the important contribution the study of dysmorphology can give in the prenatal setting.

Limitations of the study

One limitation of our study is that it is retrospective. A second partial limitation is that we did not find pathogenic CNVs and VUS in case of isolated anomalies, for this reason,

a definitive conclusion can't be extrapolated. Moreover, some subtle facial alterations and signs of dysmorphology could have escaped an invasive test and therefore not included in our analysis.

Clinical implications

Our data confirm the importance to perform an array study in case of face anomalies. Although we did not find chromosomal anomalies in the isolated subgroup, we stress the advantage of performing an array-CGH study to contribute to the diagnosis of the real prevalence of genetic anomalies, also in apparently isolated facial anomalies.

In case of associated anomalies, the array CGH allows finding new CNVs that can have an expression in the facial system and contribute to better defining syndromes and chromosomal anomalies together with a detailed anatomic scan and dysmorphology study.

7.7 URINARY ANOMALIES

Main findings

We performed a sub-analysis in a cohort of 68 cases of fetuses with urinary anomalies once two cases were excluded (1 case of pathologic QF-PCR (trisomy 21) and 1 case of failure on the array analysis). Our data showed an overall prevalence of pathogenic variants of 8.8 % and of VUS in 1.5 % of the cases.

If the urinary anomaly was associated with extra-urinary alterations, the prevalence of CNVs was higher: 11.4% compared with 4.2% in those cases with isolated urinary anomalies.

When VUS were considered, a prevalence of 4.2% of variants of unknown significance was found in the group of isolated anomalies compared with no cases of VUS among the group with associated anomalies.

In the subgroup of isolated urologic anomalies, the highest prevalence of clinically significant variants was reported in bladder anomalies (25%), being megacystis the single anomaly with the highest association with pathogenic CNVs. In the subgroup of non-isolated anomalies, the highest prevalence of pathogenic variants was found in renal hyperechogenicity (33.3%), position anomalies (22.2%) and cystic anomalies (20%).

Results in the context of what is known

Urologic malformations can affect single or multiple structures with significant variability between individuals carrying the same mutation; moreover, urologic anomalies also occur in conjunction with other associated defects indicative of known genetic syndromes. [260,261] New genomic studies allow a more comprehensive study of the molecular etiology of urologic diseases. With the advent of chromosomal microarray and next-generation sequencing, over 40 genomic disorders and 50 genes implicated in syndromic or non-syndromic forms have been found. [159] Some authors reported on array studies in fetuses with urinary system anomalies; Shaffer *et al*. reported a prevalence of clinically significant variants in 6.1% of a group of isolated genitourinary anomalies, and in 8% of cases with associated anomalies. [198] Donnelly *et al*. found an incremental yield of array over karyotype of 11.6% for renal anomalies.[63]

Data derived from the use of microarray demonstrated a consistent contribution of genetic variants in urologic malformations; these reports identified both already known and new genetic alterations, indicating a genetic heterogeneity for the urinary system's malformations. A postnatal study of more than 200 children with renal agenesis and renal dysplasia found that the most frequent genomic abnormality was 17q12 deletion, followed by 22q11.2 deletion and 1q21 deletion. [262] Another study found that up to 14% of children with renal agenesis had a pathogenic or probably pathogenic CNV. [263]

Recent data report up to a 15% diagnostic rate for cases involving parenchymal kidney defects and those cases that involve extrarenal abnormalities.[262,263] Our data, with an overall pathogenic CNVs prevalence of 8.8 %, present a slightly lower prevalence of pathogenic results compared with the one found in existing prenatal and postnatal reports.

Considering specific groups of anomalies, literature reports a higher prevalence of CNVs in the group of renal agenesis and hypoplasia/dysplasia, while ureterovesical junction obstruction and vesicoureteral reflux showed a low association with CNVs. [264]

In our study, considering isolated anomalies, we did not find any CNV when the bladder and low tract anomalies were considered, suggesting vesicoureteral reflux and junction anomalies could present a low association with genetic abnormalities; however, we encountered a pathogenic result in an isolated case of megacystis in which 16p11.2 deletion and 22q11 deletions were found. The literature reported that Chr22q11.2 locus could manifest with renal phenotypes in up to 20% to 30% cases.[265,266] Also, deletion of Chr16p11.2 is reported in association with obstructive uropathy and renal hypoplasia and dysplasia.[159]

Limitations of the study

One limitation of our work is correlated to the retrospective design of our study; moreover, since it considers only a prenatal cohort, we do not count with a follow up of those cases with mild alterations in which a prenatal array CGH study was not performed but possibly a postnatal study is available.

In addition, the low prevalence we found in the isolated group does not allow us to make definitive inferences about the contributing role of CNVs in specific renal anomalies.

Clinical implications

In our opinion, due to its incremental yield, chromosomal microarray should be strongly considered as a first-line diagnostic test for urologic anomalies once common aneuploidies are discarded with a rapid test (QF-PCR). Understanding the genetic background in case of urinary system anomalies and its contribution in the different subcategories and in those cases with complications would be essential in developing precise genetic test strategies that can guide the clinical process. A genetic diagnosis would help define the renal and extra renal phenotype and could have a critical role in preventing possible postnatal complications of a disease.

7.8 GENITAL ANOMALIES

Main findings

We performed an analysis in a cohort of 27 fetuses with genital anomalies and a prenatal array-CGH once a case with pathologic QF-PCR was excluded (one case of trisomy 13).

We found an overall prevalence of pathogenic CNV in 14.8% of the cases and VUS in 3.7 % of the total cohort.

The prevalence of abnormal array was higher when genital anomalies were associated with extragenital malformations (16% compared with no cases in the isolated group). Similarly, the prevalence of VUS was higher in those cases with associated anomalies, with a prevalence of 4% compared with no cases in the isolated anomalies group. It is to mention that most cases with genital anomalies were found in the subgroup of associated anomalies, with only 2 cases (7.4%) of isolated anomalies versus 25 cases (92.6%) in the associated group.

In the subgroup with pathogenic variants, the highest prevalence of clinically significant variants was reported in the group of testicular anomalies (42.9%), followed by abnormal genitalia (25%). The single anomaly with the highest prevalence of pathogenic CNVs was cryptorchidism (42.9%).

Results in the context of what is known

Genital defects are reported to be related to genetic mutations and variants, endocrine disorders, maternal exposure to endocrine-disrupting substances, or they can remain unexplained.[267,268] Prenatal diagnosis of genital anomalies remains inaccurate when compared with other anomalies, especially in the case of isolated genital anomalies, with a genetic study often performed after birth. [269]

When abnormal genitalia is present, an association with FGR is reported, mostly in the case of hypospadias and cryptorchidism.[270] In our study, 80% of the cases that presented

pathogenic CNVs also presented an FGR: in our case, we found cryptorchidism and ambiguous genitalia but no cases of hypospadias.

A study conducted by Fuchs *et al.* reported a higher frequency of genetic defects in a prenatal cohort of fetuses with genital defects when compared to a postnatal cohort of boys with hypospadias.[271] However, in the study, a comparison between the prevalence of genetic anomalies in prenatal and postnatal findings considering a single genital anomaly is not provided. In our series we did not found any pathogenic CNV in case of hypospadias, so we cannot make definitive conclusions regarding the performance of array in this penile malformation.

In has to be mentioned that we did not find any pathogenic result in isolated genital anomalies, and the majority of the fetuses presented associated defects. For this reason, we can make inferences only for the associated anomalies group.

We found a case of 8p23.1 duplication in a fetus presenting hypospadias associated with cardiac anomaly with a maternal transmission (gen *GATA4)*: in literature, behavioral disorders, intellectual disability, facial and cardiac alterations, congenital diaphragmatic hernia are reported; considering genitalia cryptorchidism is reported in male fetuses, but hypospadias is not reported as a frequent sign.[272,273] The 8p23.1 duplication syndrome seems rare, and it is reported in the literature with a variable gene content and not well-defined phenotype, in some cases with a very mild phenotype.[274]

In our opinion for this case the alteration detected cannot be considered as pathogenic, being *GATA4* duplications still under debate as a phenotype causative alterations.

We also found 2 cases of 15q11 deletion that presented cryptorchidism and ambiguous genitalia, respectively (one case was a BP2-BP3 deletion associated with a Prader-Willi syndrome confirmed by ME-MLPA, and the second included a deletion in the BP1-BP2 including NIPA1 and NIPA2 genes).

Unilateral or bilateral cryptorchidism is frequent in Prader Willi syndrome,[275] but not in BP1-BP2 deletion.[276] In the postnatal series, BP1-BP2 deletion is reported with a prevalence of 0.6%-1.3% when a microarray is performed.[276]

In the first case of our series, a Prader-Willi syndrome, the genital anomaly was the only structural anomaly present, apart from a fetal growth restriction.

This underlines the importance of performing an array-CGH in case of genital anomalies to rule out a genetic basis.

Limitations of the study

The main limitation of the study is related to the retrospective design. By considering only a prenatal cohort, we cannot have access to the postnatal phenotype; moreover, mild genital anomalies could have missed a diagnosis in the prenatal context, and we cannot count with a prenatal array, but for some cases, a postnatal study could be available.

In addition, since we only had two cases in the isolated group, and we did not find any clinically significant CNV or VUS, we cannot make definitive conclusions about the contributing role of CNVs in specific isolated genital anomalies.

Clinical implications

The use of array CGH in association with a standard chromosomal analysis can help in the detection of severe phenotypes of genital malformations; for these cases, a complete genetic evaluation in addition to anatomical and hormonal evaluation is of great interest because it would help to support the management of the malformation and to avoid possible pitfalls.

7.9 SKELETAL ANOMALIES

Main findings

We investigated the prevalence of array anomalies in a cohort of 48 cases of fetuses with skeletal anomalies once 2 cases with generalized fetal infection were excluded (one case each of Human Herpes virus and Zika virus).

We found an overall prevalence of pathogenic CNVs in 14.6 % of the cases and VUS in 6.2 % of the total cohort.

The prevalence of abnormal array was higher when skeletal anomalies were associated with other abnormalities and fetal growth restriction (27.3% compared with 10.8 % in the case of the isolated group). Similarly, the prevalence of VUS was higher in those cases with associated anomalies, with a prevalence of 18.2% compared with 2.7% for those cases with isolated skeletal anomalies.

In the subgroup with pathogenic variants, the highest prevalence of clinically significant variants was reported in the group of skeletal dysplasias (22.2%), followed by finger

anomalies (11.1%). The single subgroup with the highest prevalence of pathogenic CNVs was generalized skeletal dysplasias (50%).

Pathogenic CNVs in the associated group were found with the highest prevalence in the case of both upper and lower extremities anomalies (66.7% in the case of digital anomalies and 16.7% in the case of clubfoot).

Results in the context of what is known

Prenatal reports about skeletal dysplasias are scarce and difficult to compare due to different study designs, classifications, and protocols. Most of the genetic studies are often obtained postnatally. Some skeletal dysplasias present variability in penetrance, and an affected parent may be unaware of being a carrier of the condition. [277] A study by Barkova *et al*. reported 85.7% specific diagnoses based on complete studies, including molecular genetic studies. [278]

Shaffer *et al*. reported 9.1% of significant CNV when the musculoskeletal system was involved; also, when specific anomalies were studied, they found a detection rate of 13.3 % for skeletal dysplasias and 13.6% for clubfeet or hands anomalies. [198]

Recent reports have discovered many disease-causing genes with clear roles in skeletal development (e.g. genes involving *NOTCH, WNT, TGFβ, BMP* signaling). However, the roles of other genes in causing skeletal anomalies are not clear yet.[171] Recently, it has been discovered that pathogenic variants in mitochondrial proteins are associated with skeletal dysplasias, although skeletal anomalies are not common in the case of mitochondrial diseases.[279,280] Moreover, genes that do not encode proteins can cause skeletal anomalies, and mutations in regulatory sequences (outside the genes) cause

skeletal anomalies; these alterations usually provoke defects in early skeletal development and have the tendance to affect a set of bones (dysostoses). [171]

In our series, we found a higher prevalence of pathogenic variants in the case of skeletal dysplasias compared to other skeletal anomalies, confirming that in skeletal dysplasias, the genetic contribution (with different mechanisms) is high.

When clubfoot is considered, in literature, different reports argued that performing a karyotype was questionable in the case of isolated clubfoot. In a series of 51 cases, no chromosomal abnormalities were reported.[281] On the other side, when clubfoot was present with associated anomalies, the rate of chromosomal abnormalities was increased. Few authors reported on other genetic conditions in the case of a normal karyotype. In the EUROCAT study, an association with trisomy 18 and for genetic syndromes, 22q11 microdeletion and Pena-Shokeir syndrome type 1 are reported. [282]

In our series, we found a case of non-isolated clubfoot with a result in the array of Wolf-Hirschhorn syndrome due to a de novo chromosome 4p deletion. The syndrome is characterized by a typical facial feature, intellectual and developmental delay, and seizures: in addition to the typical facial appearance, multiple organ involvement has been reported including skeletal anomalies, reported depending on the authors in 21% [283] up to 60-70% of the cases.[284] Prenatal reports of Wolf-Hirschhorn syndrome are limited, and without a detailed molecular study in most cases. Deletion can arise from different mechanisms: about 50-55% of cases present a de novo 4p deletion, 40-45% result from an unbalanced translocation (de novo or inherited from a parent with a balanced translocation), the remaining 5% are complex rearrangements.[285] Karyotype presents a 50-60% sensitivity, whereas CMA approaches 100% in recent reports.[283]

Limitations of the study

Our series has a retrospective design, and we do not count on the postnatal follow up of most cases. Moreover, only in some cases in which the suspect of a monogenic condition was high, and the array gave a normal result, an oriented panel for skeletal dysplasias was run.

Clinical implications

The use of array CGH in cases of a suspect of skeletal anomaly would help in an early diagnosis and lower the number of genetic studies performed postnatally. Moreover, it widens the spectrum of possible diagnoses, providing extreme important information for prenatal parental counselling on the present and future gestations.

7.10 HIDROPS FETALIS

Main findings

We studied the prevalence of array anomalies in 23 cases of fetuses with hydrops once 2 cases were excluded (one case due to Human herpes virus infection and one case of trisomy 18).

We encountered an overall prevalence of pathogenic CNVs in 8.7 % of the cases and VUS in 8.7% of the total cohort.

The prevalence of abnormal array was only slightly higher when other anomalies were associated with hydrops (9.1% compared with 8.3% in the case of isolated hydrops). No VUS was found in the case of isolated hydrops, compared to a prevalence of 18.2 % in non-isolated hydrops.

Results in the context of what is known

In prenatal series, the most common cause of NIHF is aneuploidy, especially when diagnosed at early stages of gestation.[286,287] Hydrops can be present as a primary feature or secondary to other anomalies mainly: cardiovascular (17-35%), chromosomal (7-16%), hematologic (4-12%), infectious (5-7%), thoracic (6%), twin-twin transfusion (3-10%), urinary tract abnormalities (2-3%), gastrointestinal (0.5-4%), lymphatic dysplasia (5-6%), placental anomalies (2-3%), skeletal dysplasias (3-4%), and syndromic (3-4%).[185] Limited data are published on the yield of microarray for non-immune hydrops, but it might be in the range of 6-14 %.[185,187,288]

It is unclear also the impact the use of CMA has over karyotype in diagnosing more genetic variants as etiologic causes for NIHF; this probably is due to the highly heterogeneous group of genetic etiologies of NIHF, including single-gene disorders (not detectable by CMA).[289]

Regarding the array's contribution, Shaffer *et al*. reported an overall detection rate of 8.0% by CMA (7% in case of isolated hydrops versus 9% in those cases with other associated anomalies).[198] A report of Sparks *et al*. found a genetic etiology of the NIHF in 25% of the cohort with genetic testing using CMA and/or karyotype. Another group found an incremental yield of CMA of 4.2% for pathogenic variants and 4.2% for variants of unknown significance. [290]Our group found similar results with no differences between pathogenic and unknown significant variants. Similarly to our findings, Deng et al. did not find significant differences between isolated and associated hydrops in terms of pathogenic CNVs. [290]

Limitations of the study

It is known that many genetic syndromes (metabolic disorders and rare autosomal recessive conditions) are caused by variants that CMA does not detect, so we miss conclusive data from those cases of hydrops in which the genetic content cannot be detected by karyotype or microarray.

Clinical implications

Most of the existing literature consists of case reports and small series focusing on specific genetic diseases, and the actual frequency of each underlying cause remains uncertain. We considered a series of non-immune hydrops fetalis, isolated or not, and from this, we studied the possible genetic contribution. Our study includes a recent cohort of cases, all of which were assessed by CMA, one of the most recent genetic testings for prenatal diagnosis. We stress the importance of excluding a genetic abnormality using an array platform. It widens the number of chromosomal abnormalities that can be diagnosed once common aneuploidies are discarded, and allows more detailed counselling to the families about the prognosis and the possibility of recurrence in future gestations.

PART 8

CONCLUSIONS

8 CONCLUSIONS

8.1 PREVALENCE OF PATHOGENIC CNV AND VUS IN THE PRESENCE OF FETAL STRUCTURAL ANOMALIES

The results of our study provide strong evidence towards performing an array test in case of ultrasound anomalies in different systems: central nervous system, cardiac thoracic, gastrointestinal, facial, urinary, genital, skeletal and hydrops.

The overall prevalence of pathogenic CNV was 8 %, while the one of VUS was 4.3%.

8.2 PREVALENCE OF PATHOGENIC CNV AND VUS IN SPECIFIC ORGANS AND/OR SYSTEMS

The stronger association with genetic anomalies detected by array-CGH was found in the case of facial anomalies, followed by genital and skeletal anomalies. The lowest prevalence of CNVs was detected in gastrointestinal defects followed by central nervous system anomalies.

Regarding variants of unknown significance, the stronger association was found in case of fetal non-immune hydrops and central nervous system anomalies, while the lowest was found in case of urinary, cardiac, and genital anomalies.

8.3 PREVALENCE OF PATHOGENIC CNV AND VUS IN SPECIFIC SUBGROUPS OF ANOMALIES

Considering the specific anomalies with the highest association with pathogenic CNV the results are the following:

- For CNS anomalies the highest percentage of pathogenic CNV is found in case of posterior fossa abnormalities.
- For cardiac anomalies the highest association is found in case of left and right heart defects, septal defects and conotruncal anomalies.
- For thoracic anomalies the highest prevalence is found in case of hydrothorax and congenital diaphragmatic hernia.
- For gastrointestinal anomalies the highest prevalence is found in those cases with abdominal wall defects.
- For facial anomalies the highest prevalence is detected in those cases with a diagnosis of facial dysmorphology as it can be a sign of a more complex syndrome. To perform an array test is of special importance in case of all kinds of facial anomalies due to the high prevalence of pathogenic CNVs in all the subgroups of facial anomalies (when the associated anomalies group is considered). No pathogenic CNVs and VUS were found to be a possible cause of facial defects in case of isolated facial anomalies.
- For urinary anomalies the highest association is found in cases of renal position anomalies, renal hyperecogenicity and bladder anomalies; the prevalence of variants of unknown significance was higher in isolated cases when compared with those with associated anomalies.
- For genital anomalies the subgroup of defects that presented the highest prevalence of pathogenic variants was the one of testicular anomalies.
- For skeletal anomalies the highest prevalence of pathogenic variants is found in case of skeletal dysplasias and digital anomalies. However, it must be underlined

that for skeletal system many genetic causes are beyond the resolutions of CMA (such as monogenic diseases).

- For non-immune hydrops fetalis the prevalence of pathogenic CNVs and VUS is similar. As reported for skeletal anomalies, for hydrops fetalis, a considerable percentage of etiologic cause can be missed due to single gene disorders that chromosomal microarray is not able to detect.

8.4 PREVALENCE OF PATHOGENIC CNV AND VUS IN ISOLATED ANOMALIES VERSUS IN THE PRESENCE OF ASSOCIATED ANOMALIES

The prevalence of pathogenic CNVs in case of associated anomalies was higher than in those cases of isolated anomalies for all the systems considered.

The systems presenting higher prevalence of pathogenic variants if the anomaly was isolated to only one system were skeletal anomalies with a prevalence of 10.8%, followed by cardiac anomalies with a prevalence of 10.4%, and fetal non-immune hydrops with a prevalence of 8.3%. The lowest prevalence of pathogenic array results in isolated anomalies was found in gastrointestinal anomalies (2.1%) and facial and genital system with no pathogenic CNV detected.

If VUS are considered, in isolated anomalies, the highest prevalence is found in central nervous system (7.7%) and thoracic anomalies (5.3%), while the lowest was detected in facial and genital anomalies and in case of fetal hydrops with no cases of VUS.

8.5 SPECIFIC PATTERNS OF CNVS

Regarding the presence of specific genetic patterns and involvement of genes, in our series we found SOX3 duplication in a male fetus affected with spina bifida; SOX3 duplication has been recently associated with neural tube defects.

In the thoracic system we found an association of left-sided CDH with deletion in Xq26.2 (which include GPC3 gene, associated with Simpson-Golabi-Behmel Syndrome).

In urinary system we confirmed the association of Chr 22q11.2 locus alterations and deletion of Chr 16p11.2 with anomalous renal phenotypes.

Considering genitalia, we also found an association of 15q11 Prader-Willi associated deletion with abnormal genitalia (cryptorchidism and ambiguous genitalia), being the other case, a BP1-BP2 deletion including NIPA1 and NIPA2 gene.

PART 9

REFERENCES

9 REFERENCES

1. Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, Savage M, Platt LD, Saltzman D, Grobman WA, Klugman S, Scholl T, Simpson JL, McCall K, Aggarwal VS, Bunke B, Nahum O, Patel A, Lamb AN, Thom EA, Beaudet AL, Ledbetter DH, Shaffer LG, Jackson L. Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis. *N Engl J Med*. 2012;367(23):2175-2184. doi:10.1056/NEJMoa1203382

2. Bui TH, Vetro A, Zuffardi O, Shaffer LG. Current controversies in prenatal diagnosis 3: is conventional chromosome analysis necessary in the post-array CGH era?: CURRENT CONTROVERSIES IN PRENATAL DIAGNOSIS 3. *Prenat Diagn*. 2011;31(3):235-243. doi:10.1002/pd.2722

3. Miller DT, Adam MP, Aradhya S, Biesecker LG, Brothman AR, Carter NP, Church DM, Crolla JA, Eichler EE, Epstein CJ, Faucett WA, Feuk L, Friedman JM, Hamosh A, Jackson L, Kaminsky EB, Kok K, Krantz ID, Kuhn RM, Lee C, Ostell JM, Rosenberg C, Scherer SW, Spinner NB, Stavropoulos DJ, Tepperberg JH, Thorland EC, Vermeesch JR, Waggoner DJ, Watson MS, Martin CL, Ledbetter DH. Consensus Statement: Chromosomal Microarray Is a First-Tier Clinical Diagnostic Test for Individuals with Developmental Disabilities or Congenital Anomalies. *Am J Hum Genet*. 2010;86(5):749-764. doi:10.1016/j.ajhg.2010.04.006

4. Committee Opinion No.682: Microarrays and Next-Generation Sequencing Technology: The Use of Advanced Genetic Diagnostic Tools in Obstetrics and Gynecology. *Obstet Gynecol*. 2016;128(6):e262-e268. doi:10.1097/AOG.0000000000001817

5. Gil MM, Molina FS, Rodríguez-Fernández M, Delgado JL, Carrillo MP, Jani J, Plasencia W, Stratieva V, Maíz N, Carretero P, Lismonde A, Chaveeva P, Burgos J, Santacruz B, Zamora J, De Paco Matallana C. New approach for estimating risk of miscarriage after chorionic villus sampling. *Ultrasound Obstet Gynecol*. 2020;56(5):656-663. doi:10.1002/uog.22041

6. Royal College of Obstetricians & Gynaecologists. Royal College of Obstetricians & Gynaecologists. Amniocentesis and Chorionic Villus Sampling. Green-top Guideline No. 8, June 2010. *Amniocentesis Chorionic Villus Sampl*. Published online June 2010.

7. Jackson LG, Zachary JM, Fowler SE, Desnick RJ, Golbus MS, Ledbetter DH, Mahoney MJ, Pergament E, Simpson JL, Black S, Wapner RJ. A Randomized Comparison of Transcervical and Transabdominal Chorionic-Villus Sampling. *N Engl J Med*. 1992;327(9):594-598. doi:10.1056/NEJM199208273270903

8. Akolekar. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2015;45(1):16-26. doi:10.1002/uog.14636

9. Wulff CB, Gerds TA, Rode L, Ekelund CK, Petersen OB, Tabor A, the Danish Fetal Medicine Study Group. Risk of fetal loss associated with invasive testing following combined first-trimester screening for Down syndrome: a national cohort of 147 987 singleton pregnancies: Procedure-related risk of fetal loss. *Ultrasound Obstet Gynecol*. 2016;47(1):38-44. doi:10.1002/uog.15820

10. ACOG Practice Bulletin No. 88: Invasive Prenatal Testing for Aneuploidy. *Obstet Gynecol*. 2007;110(6):1459-1467. doi:10.1097/01.AOG.0000291570.63450.44

11. Ghi T, Sotiriadis A, Calda P, Da Silva Costa F, Raine-Fenning N, Alfirevic Z, McGillivray G, International Society of Ultrasound in Obstetrics and Gynecology (ISUOG). ISUOG Practice Guidelines: invasive procedures for prenatal diagnosis: ISUOG Guidelines. *Ultrasound Obstet Gynecol*. 2016;48(2):256-268. doi:10.1002/uog.15945

12. Salomon LJ, Sotiriadis A, Wulff CB, Odibo A, Akolekar R. Risk of miscarriage following amniocentesis or chorionic villus sampling: systematic review of literature and updated meta-analysis. *Ultrasound Obstet Gynecol*. 2019;54(4):442-451. doi:10.1002/uog.20353

13. Philip J, Silver RK, Wilson RD, Thom EA, Zachary JM, Mohide P, Mahoney MJ, Simpson JL, Platt LD, Pergament E, Hershey D, Filkins K, Johnson A, Shulman LP, Bang J, MacGregor S, Smith JR, Shaw D, Wapner RJ, Jackson LG. Late First-Trimester Invasive Prenatal Diagnosis: Results of an International Randomized Trial: *Obstet Gynecol*. 2004;103(6):1164-1173. doi:10.1097/01.AOG.0000128049.73556.fb

14. Beta J, Lesmes-Heredia C, Bedetti C,, Akolekar R. Risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review of the literature. *Minerva Ginecol*. 2018;70(2):215-219. doi:10.23736/S0026-4784.17.04178-8

15. Alfirevic Z, Navaratnam K, Mujezinovic F. Amniocentesis and chorionic villus sampling for prenatal diagnosis. Cochrane Pregnancy and Childbirth Group, ed. *Cochrane Database Syst Rev*. Published online September 4, 2017. doi:10.1002/14651858.CD003252.pub2

16. Cruz-Lemini M, Parra-Saavedra M, Borobio V, Bennasar M, Goncé A, Martínez JM, Borrell A. How to perform an amniocentesis. *Ultrasound Obstet Gynecol*. 2014;44(6):727-731. doi:10.1002/uog.14680

17. Shaffer LG. A cytogeneticist's perspective on genomic microarrays. *Hum Reprod Update*. 2004;10(3):221-226. doi:10.1093/humupd/dmh022

18. Riggs ER, Andersen EF, Cherry AM, Kantarci S, Kearney H, Patel A, Raca G, Ritter DI, South ST, Thorland EC, Pineda-Alvarez D, Aradhya S, Martin CL. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). *Genet Med*. 2020;22(2):245-257. doi:10.1038/s41436-019-0686-8

19. Nowakowska B. Clinical interpretation of copy number variants in the human genome. *J Appl Genet*. 2017;58(4):449-457. doi:10.1007/s13353-017-0407-4

20. Henrichsen CN, Chaignat E, Reymond A. Copy number variants, diseases and gene expression. *Hum Mol Genet*. 2009;18(R1):R1-R8. doi:10.1093/hmg/ddp011

21. Tuzun E, Sharp AJ, Bailey JA, Kaul R, Morrison VA, Pertz LM, Haugen E, Hayden H, Albertson D, Pinkel D, Olson MV, Eichler EE. Fine-scale structural variation of the human genome. *Nat Genet*. 2005;37(7):727-732. doi:10.1038/ng1562

22. Levy B, Wapner R. Prenatal diagnosis by chromosomal microarray analysis. *Fertil Steril*. 2018;109(2):201-212. doi:10.1016/j.fertnstert.2018.01.005

23. Redon R, Ishikawa S, Fitch KR, Feuk L, Perry GH, Andrews TD, Fiegler H, Shapero MH, Carson AR, Chen W, Cho EK, Dallaire S, Freeman JL, González JR, Gratacòs M, Huang J, Kalaitzopoulos D, Komura D, MacDonald JR, Marshall CR, Mei R, Montgomery L, Nishimura K, Okamura K, Shen F, Somerville MJ, Tchinda J, Valsesia A, Woodwark C, Yang F, Zhang J, Zerjal T, Zhang J, Armengol L, Conrad DF, Estivill X, Tyler-Smith C, Carter NP, Aburatani H, Lee C, Jones KW, Scherer SW, Hurles ME. Global variation in copy number in the human genome. *Nature*. 2006;444(7118):444-454. doi:10.1038/nature05329

24. Fanciulli M, Petretto E, Aitman T. Gene copy number variation and common human disease. *Clin Genet*. 2010;77(3):201-213. doi:10.1111/j.1399-0004.2009.01342.x

25. The Wellcome Trust Case Control Consortium, Conrad DF, Pinto D, Redon R, Feuk L, Gokcumen O, Zhang Y, Aerts J, Andrews TD, Barnes C, Campbell P, Fitzgerald T, Hu M, Ihm CH, Kristiansson K, MacArthur DG, MacDonald JR, Onyiah I, Pang AWC, Robson S, Stirrups K, Valsesia A, Walter K, Wei J, Tyler-Smith C, Carter NP, Lee C, Scherer SW, Hurles ME. Origins and functional impact of copy number variation in the human genome. *Nature*. 2010;464(7289):704-712. doi:10.1038/nature08516

26. Girirajan S, Rosenfeld JA, Coe BP, Parikh S, Friedman N, Goldstein A, Filipink RA, McConnell JS, Angle B, Meschino WS, Nezarati MM, Asamoah A, Jackson KE, Gowans GC, Martin JA, Carmany EP, Stockton DW, Schnur RE, Penney LS, Martin DM, Raskin S, Leppig K, Thiese H, Smith R, Aberg E, Niyazov DM, Escobar LF, El-Khechen D, Johnson KD, Lebel RR, Siefkas K, Ball S, Shur N, McGuire M, Brasington CK, Spence JE, Martin LS, Clericuzio C, Ballif BC, Shaffer LG, Eichler EE. Phenotypic heterogeneity of genomic disorders and rare copy-number variants. *N Engl J Med*. 2012;367(14):1321-1331. doi:10.1056/NEJMoa1200395

27. Stankiewicz P, Inoue K, Bi W, Walz K, Park SS, Kurotaki N, Shaw CJ, Fonseca P, Yan J, Lee JA, Khajavi M, Lupski JR. Genomic Disorders: Genome Architecture Results in Susceptibility to DNA Rearrangements Causing Common Human Traits. *Cold Spring Harb Symp Quant Biol*. 2003;68(0):445-454. doi:10.1101/sqb.2003.68.445

28. The 1000 Genomes Project Consortium. A map of human genome variation from population-scale sequencing. *Nature*. 2010;467(7319):1061-1073. doi:10.1038/nature09534

29. Landrum MJ, Lee JM, Benson M, Brown G, Chao C, Chitipiralla S, Gu B, Hart J, Hoffman D, Hoover J, Jang W, Katz K, Ovetsky M, Riley G, Sethi A, Tully R, Villamarin-Salomon R, Rubinstein W, Maglott DR. ClinVar: public archive of interpretations of clinically relevant variants. *Nucleic Acids Res*. 2016;44(D1):D862-D868. doi:10.1093/nar/gkv1222

30. Firth HV, Richards SM, Bevan AP, Clayton S, Corpas M, Rajan D, Vooren SV, Moreau Y, Pettett RM, Carter NP. DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. *Am J Hum Genet*. 2009;84(4):524-533. doi:10.1016/j.ajhg.2009.03.010

31. McGowan-Jordan J, Hastings RJ, Moore S S Karger (Bazylea). *ISCN 2020: An International System for Human Cytogenetic Nomenclature (2020)*. Karger; 2020.

32. Stark Z, Gillam L, Walker SP, McGillivray G. Ethical controversies in prenatal microarray. *Curr Opin Obstet Gynecol*. 2013;25(2):133-137. doi:10.1097/GCO.0b013e32835ebb67

33. Hillman SC, Pretlove S, Coomarasamy A, Mcmullan DJ, Davison EV, Maher ER, Kilby MD. Additional information from array comparative genomic hybridization technology over conventional karyotyping in prenatal diagnosis: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2011;37(1):6-14. doi:10.1002/uog.7754

34. Green J, Hewison J, Bekker H, Bryant L, Cuckle H. Psychosocial aspects of genetic screening of pregnant women and newborns: a systematic review. *Health Technol Assess*. 2004;8(33). doi:10.3310/hta8330

35. Bernhardt BA, Soucier D, Hanson K, Savage MS, Jackson L, Wapner RJ. Women's experiences receiving abnormal prenatal chromosomal microarray testing results. *Genet Med*. 2013;15(2):139-145. doi:10.1038/gim.2012.113

36. Levenson D. Array CGH increasingly used in prenatal and postnatal testing. *Am J Med Genet A*. 2012;158A(3):viii-ix. doi:10.1002/ajmg.a.35281

37. McGillivray G, Rosenfeld JA, McKinlay Gardner RJ, Gillam LH. Genetic counselling and ethical issues with chromosome microarray analysis in prenatal testing: Genetic counselling and ethical Issues with prenatal CMA. *Prenat Diagn*. 2012;32(4):389-395. doi:10.1002/pd.3849

38. Faas BH, Feenstra I, Eggink AJ, Kooper AJ, Pfundt R, van Vugt JM, de Leeuw N. Non-targeted whole genome 250K SNP array analysis as replacement for karyotyping in fetuses with structural ultrasound anomalies: evaluation of a one-year experience: Non-targeted whole genome 250K SNP array analysis in prenatal diagnosis. *Prenat Diagn*. 2012;32(4):362-370. doi:10.1002/pd.2948

39. Vanakker O, Vilain C, Janssens K, Van der Aa N, Smits G, Bandelier C, Blaumeiser B, Bulk S, Caberg JH, De Leener A, De Rademaeker M, de Ravel T, Desir J, Destree A, Dheedene A, Gaillez S, Grisart B, Hellin AC, Janssens S, Keymolen K, Menten B, Pichon B, Ravoet M, Revencu N, Rombout S, Staessens C, Van Den Bogaert A, Van Den Bogaert K, Vermeesch JR, Kooy F, Sznajer Y, Devriendt K. Implementation of genomic arrays in prenatal diagnosis: The Belgian approach to meet the challenges. *Eur J Med Genet*. 2014;57(4):151-156. doi:10.1016/j.ejmg.2014.02.002

40. Muys J, Blaumeiser B, Jacquemyn Y, Bandelier C, Brison N, Bulk S, Chiarappa P, Courtens W, De Leener A, De Rademaeker M, Désir J, Destrée A, Devriendt K, Dheedene A, Fieuw A, Fransen E, Gatot J, Holmgren P, Jamar M, Janssens S, Keymolen K, Lederer D, Menten B, Meuwissen M, Parmentier B, Pichon B, Rombout S, Sznajer Y, Van Den Bogaert A, Van Den Bogaert K, Vanakker O, Vermeesch J, Janssens K. The Belgian MicroArray Prenatal (BEMAPRE) database: A systematic nationwide repository of fetal genomic aberrations. *Prenat Diagn*. 2018;38(13):1120-1128. doi:10.1002/pd.5373

41. https://www.beshg.be/.

42. Crolla JA, Wapner R, Van Lith JMM. Controversies in prenatal diagnosis 3: should everyone undergoing invasive testing have a microarray?: Controversies in prenatal diagnosis 3. *Prenat Diagn*. 2014;34(1):18-22. doi:10.1002/pd.4287

43. Royal College of Pathologists, PUB LTRC of P. Recommendations for the use of chromosome microarray in pregnancy. 2015;(PUB 290615).

44. Royal Australian and New Zealand College of Obstetricians, and Gynaecologists. Prenatal screening and diagnosis of chromosomal and genetic conditions in the fetus in pregnancy. 2016;C-Obs 59.

45. Karampetsou E, Morrogh D, Chitty L. Microarray Technology for the Diagnosis of Fetal Chromosomal Aberrations: Which Platform Should We Use? *J Clin Med*. 2014;3(2):663-678. doi:10.3390/jcm3020663

46. Ylstra B. BAC to the future! or oligonucleotides: a perspective for micro array comparative genomic hybridization (array CGH). *Nucleic Acids Res*. 2006;34(2):445-450. doi:10.1093/nar/gkj456

47. Bignell GR. High-Resolution Analysis of DNA Copy Number Using Oligonucleotide Microarrays. *Genome Res*. 2004;14(2):287-295. doi:10.1101/gr.2012304

48. Papenhausen P, Schwartz S, Risheg H, Keitges E, Gadi I, Burnside RD, Jaswaney V, Pappas J, Pasion R, Friedman K, Tepperberg J. UPD detection using homozygosity profiling with a SNP genotyping microarray. *Am J Med Genet A*. 2011;155(4):757-768. doi:10.1002/ajmg.a.33939

49. ClinGen. https://www.clinicalgenome.org/.

50. https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000205.v6.p2.

51. Vissers LELM, de Vries BBA, Veltman JA. Genomic microarrays in mental retardation: from copy number variation to gene, from research to diagnosis. *J Med Genet*. 2010;47(5):289-297. doi:10.1136/jmg.2009.072942

52. Callaway JLA, Shaffer LG, Chitty LS, Rosenfeld JA, Crolla JA. The clinical utility of microarray technologies applied to prenatal cytogenetics in the presence of a normal conventional karyotype: a review of the literature. *Prenat Diagn*. 2013;33(12):1119-1123. doi:10.1002/pd.4209

53. Shaffer LG, Dabell MP, Fisher AJ, Coppinger J, Bandholz AM, Ellison JW, Ravnan JB, Torchia BS, Ballif BC, Rosenfeld JA. Experience with microarray-based comparative genomic hybridization for prenatal diagnosis in over 5000 pregnancies. *Prenat Diagn*. 2012;32(10):976-985. doi:10.1002/pd.3945

54. Lovrecic L, Remec ZI, Volk M, Rudolf G, Writzl K, Peterlin B. Clinical utility of array comparative genomic hybridisation in prenatal setting. *BMC Med Genet*. 2016;17(1):81. doi:10.1186/s12881-016-0345-8

55. Srebniak MI, Joosten M, Knapen MFCM, Arends LR, Polak M, van Veen S, Go ATJI, Van Opstal D. Frequency of submicroscopic chromosomal aberrations in pregnancies without increased risk for structural chromosomal aberrations: systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2018;51(4):445-452. doi:10.1002/uog.17533

56. Krishnaswamy S, Subramaniam K, Ramachandran P, Indran T, Abdul Aziz J. Delayed fathering and risk of mental disorders in adult offspring. *Early Hum Dev*. 2011;87(3):171-175. doi:10.1016/j.earlhumdev.2010.12.004

57. Kong A, Frigge ML, Masson G, Besenbacher S, Sulem P, Magnusson G, Gudjonsson SA, Sigurdsson A, Jonasdottir A, Jonasdottir A, Wong WSW, Sigurdsson G, Walters GB, Steinberg S, Helgason H, Thorleifsson G, Gudbjartsson DF, Helgason A, Magnusson OTh, Thorsteinsdottir U, Stefansson K. Rate of de novo mutations and the

importance of father's age to disease risk. *Nature*. 2012;488(7412):471-475. doi:10.1038/nature11396

58. Buizer-Voskamp JE, Blauw HM, Boks MPM, van Eijk KR, Veldink JH, Hennekam EAM, Vorstman JAS, Mulder F, Tiemeier H, Uitterlinden AG, Kiemeney LA, van den Berg LH, Kahn RS, Sabatti C, Ophoff RA. Increased paternal age and the influence on burden of genomic copy number variation in the general population. *Hum Genet*. 2013;132(4):443-450. doi:10.1007/s00439-012-1261-4

59. Bornstein E, Berger S, Cheung S, Maliszewski K, Patel A, Pursley A, Lenchner E, Bacino C, Beaudet A, Divon M. Universal Prenatal Chromosomal Microarray Analysis: Additive Value and Clinical Dilemmas in Fetuses with a Normal Karyotype. *Am J Perinatol*. 2016;34(04):340-348. doi:10.1055/s-0036-1586501

60. Wapner RJ, Martin CL, Levy B, Ballif BC, Eng CM, Zachary JM, Savage M, Platt LD, Saltzman D, Grobman WA, Klugman S, Scholl T, Simpson JL, McCall K, Aggarwal VS, Bunke B, Nahum O, Patel A, Lamb AN, Thom EA, Beaudet AL, Ledbetter DH, Shaffer LG, Jackson L. Chromosomal Microarray versus Karyotyping for Prenatal Diagnosis. *N Engl J Med*. 2012;367(23):2175-2184. doi:10.1056/NEJMoa1203382

61. Shaffer LG, Rosenfeld JA, Dabell MP, Coppinger J, Bandholz AM, Ellison JW, Ravnan JB, Torchia BS, Ballif BC, Fisher AJ. Detection rates of clinically significant genomic alterations by microarray analysis for specific anomalies detected by ultrasound. *Prenat Diagn*. 2012;32(10):986-995. doi:10.1002/pd.3943

62. Hillman SC, McMullan DJ, Hall G, Togneri FS, James N, Maher EJ, Meller CH, Williams D, Wapner RJ, Maher ER, Kilby MD. Use of prenatal chromosomal microarray:

prospective cohort study and systematic review and meta-analysis: Prenatal CMA: cohort study and systematic review. *Ultrasound Obstet Gynecol*. 2013;41(6):610-620. doi:10.1002/uog.12464

63. Donnelly JC, Platt LD, Rebarber A, Zachary J, Grobman WA, Wapner RJ. Association of Copy Number Variants With Specific Ultrasonographically Detected Fetal Anomalies. *Obstet Gynecol*. 2014;124(1):83-90. doi:10.1097/AOG.0000000000000336

64. Bernier PL, Stefanescu A, Samoukovic G, Tchervenkov CI. The Challenge of Congenital Heart Disease Worldwide: Epidemiologic and Demographic Facts. *Semin Thorac Cardiovasc Surg Pediatr Card Surg Annu*. 2010;13(1):26-34. doi:10.1053/j.pcsu.2010.02.005

65. Song MS, Hu A, Dyhamenahali U, Chitayat D, Winsor EJT, Ryan G, Smallhorn J, Barrett J, Yoo SJ, Hornberger LK. Extracardiac lesions and chromosomal abnormalities associated with major fetal heart defects: comparison of intrauterine, postnatal and postmortem diagnoses. *Ultrasound Obstet Gynecol*. 2009;33(5):552-559. doi:10.1002/uog.6309

66. Jenkins KJ, Correa A, Feinstein JA, Botto L, Britt AE, Daniels SR, Elixson M, Warnes CA, Webb CL. Noninherited Risk Factors and Congenital Cardiovascular Defects: Current Knowledge: A Scientific Statement From the American Heart Association Council on Cardiovascular Disease in the Young: *Endorsed by the American Academy of Pediatrics*. *Circulation*. 2007;115(23):2995-3014. doi:10.1161/CIRCULATIONAHA.106.183216

67. AIUM Practice Guideline for the Performance of Obstetric Ultrasound Examinations. *J Ultrasound Med*. 2013;32(6):1083-1101. doi:10.7863/jum.2013.32.6.1083

68. Mademont-Soler I, Morales C, Soler A, MartÍnez-Crespo JM, Shen Y, Margarit E, Clusellas N, Obón M, Wu BL, Sánchez A. Prenatal diagnosis of chromosomal abnormalities in fetuses with abnormal cardiac ultrasound findings: evaluation of chromosomal microarray-based analysis: Prenatal CMA for CHD. *Ultrasound Obstet Gynecol*. 2013;41(4):375-382. doi:10.1002/uog.12372

69. Hopkins MK, Dugoff L, Kuller JA. Congenital Heart Disease: Prenatal Diagnosis and Genetic Associations. *Obstet Gynecol Surv*. 2019;74(8):497-503. doi:10.1097/OGX.0000000000000702

70. Asim A, Kumar A, Muthuswamy S, Jain S, Agarwal S. "Down syndrome: an insight of the disease." *J Biomed Sci*. 2015;22(1):41. doi:10.1186/s12929-015-0138-y

71. Bergstro m S, Carr H, Petersson G, Stephansson O, Bonamy AKE, Dahlstro m A, Halvorsen CP, Johansson S. Trends in Congenital Heart Defects in Infants With Down Syndrome. *PEDIATRICS*. 2016;138(1):e20160123-e20160123. doi:10.1542/peds.2016-0123

72. Freeman SB, Taft LF, Dooley KJ, Allran K, Sherman SL, Hassold TJ, Khoury MJ, Saker DM. Population-based study of congenital heart defects in Down syndrome. *Am J Med Genet*. 1998;80(3):213-217.

73. Cleves MA, Hobbs CA, Cleves PA, Tilford JM, Bird TM, Robbins JM. Congenital defects among liveborn infants with Down syndrome. *Birt Defects Res A Clin Mol Teratol*. 2007;79(9):657-663. doi:10.1002/bdra.20393

74. Parker SE, Mai CT, Canfield MA, Rickard R, Wang Y, Meyer RE, Anderson P, Mason CA, Collins JS, Kirby RS, Correa A, for the National Birth Defects Prevention Network. Updated national birth prevalence estimates for selected birth defects in the United States, 2004-2006. *Birt Defects Res A Clin Mol Teratol*. 2010;88(12):1008-1016. doi:10.1002/bdra.20735

75. Musewe NN, Alexander DJ, Teshima I, Smallhorn JF, Freedom RM. Echocardiographic evaluation of the spectrum of cardiac anomalies associated with trisomy 13 and trisomy 18. *J Am Coll Cardiol*. 1990;15(3):673-677. doi:10.1016/0735-1097(90)90644-5

76. Petry P, Polli JB, Mattos VF, Rosa RCM, Zen PRG, Graziadio C, Paskulin GA, Rosa RFM. Clinical features and prognosis of a sample of patients with trisomy 13 (Patau syndrome) from Brazil. *Am J Med Genet A*. 2013;161(6):1278-1283. doi:10.1002/ajmg.a.35863

77. Cramer JW, Bartz PJ, Simpson PM, Zangwill SD. The Spectrum of Congenital Heart Disease and Outcomes After Surgical Repair Among Children With Turner Syndrome: A Single-Center Review. *Pediatr Cardiol*. 2014;35(2):253-260. doi:10.1007/s00246-013-0766-5

78. Kim HK, Gottliebson W, Hor K, Backeljauw P, Gutmark-Little I, Salisbury SR, Racadio JM, Helton-Skally K, Fleck R. Cardiovascular Anomalies in Turner Syndrome:

Spectrum, Prevalence, and Cardiac MRI Findings in a Pediatric and Young Adult Population. *Am J Roentgenol*. 2011;196(2):454-460. doi:10.2214/AJR.10.4973

79. Bondy C, Bakalov VK, Cheng C, Olivieri L, Rosing DR, Arai AE. Bicuspid aortic valve and aortic coarctation are linked to deletion of the X chromosome short arm in Turner syndrome. *J Med Genet*. 2013;50(10):662-665. doi:10.1136/jmedgenet-2013-101720

80. Mcdonald-Mcginn DM, Tonnesen MK, Laufer-Cahana A, Finucane B, Driscoll DA, Emanuel BS, Zackai EH. Phenotype of the 22q11.2 deletion in individuals identified through an affected relative: Cast a wide FISHing net! *Genet Med*. 2001;3(1):23-29. doi:10.1097/00125817-200101000-00006

81. Iserin L, de Lonlay P, Viot G, Sidi D, Kachaner J, Munnich A, Lyonnet S, Vekemans M, Bonnet D. Prevalence of the microdeletion 22q11 in newborn infants with congenital conotruncal cardiac anomalies. *Eur J Pediatr*. 1998;157(11):881-884. doi:10.1007/s004310050959

82. Botto LD, May K, Fernhoff PM, Correa A, Coleman K, Rasmussen SA, Merritt RK, O'Leary LA, Wong LY, Elixson EM, Mahle WT, Campbell RM. A Population-Based Study of the 22q11.2 Deletion: Phenotype, Incidence, and Contribution to Major Birth Defects in the Population. *PEDIATRICS*. 2003;112(1):101-107. doi:10.1542/peds.112.1.101

83. Hartman RJ, Rasmussen SA, Botto LD, Riehle-Colarusso T, Martin CL, Cragan JD, Shin M, Correa A. The Contribution of Chromosomal Abnormalities to Congenital Heart Defects: A Population-Based Study. *Pediatr Cardiol*. 2011;32(8):1147-1157. doi:10.1007/s00246-011-0034-5

84. Rickert-Sperling S, et al. *Congenital Heart Diseases: The Broken Heart: Clinical Features, Human Genetics AndMolecular Pathways.* Springer-Verlag; 2016.

85. Jansen FAR, Blumenfeld YJ, Fisher A, Cobben JM, Odibo AO, Borrell A, Haak MC. Array comparative genomic hybridization and fetal congenital heart defects: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2015;45(1):27-35. doi:10.1002/uog.14695

86. Sukenik-Halevy R, Sukenik S, Koifman A, Alpert Y, Hershkovitz R, Levi A, Biron-Shental T. Clinical aspects of prenatally detected congenital heart malformations and the yield of chromosomal microarray analysis: Chromosomal microarray for congenital heart malformations. *Prenat Diagn*. 2016;36(13):1185-1191. doi:10.1002/pd.4954

87. Liao C, Li R, Fu F, Xie G, Zhang Y, Pan M, Li J, Li D. Prenatal diagnosis of congenital heart defect by genome-wide high-resolution SNP array: Chromosome microarray analysis of CHD. *Prenat Diagn*. 2014;34(9):858-863. doi:10.1002/pd.4383

88. Yan Y, Wu Q, Zhang L, Wang X, Dan S, Deng D, Sun L, Yao L, Ma Y, Wang L. Detection of submicroscopic chromosomal aberrations by array-based comparative genomic hybridization in fetuses with congenital heart disease: aCGH detects fetuses with CHD. *Ultrasound Obstet Gynecol*. 2014;43(4):404-412. doi:10.1002/uog.13236

89. Zhu X, Li J, Ru T, Wang Y, Xu Y, Yang Y, Wu X, Cram DS, Hu Y. Identification of copy number variations associated with congenital heart disease by chromosomal microarray analysis and next-generation sequencing: Prenatal genetic diagnosis of CHD. *Prenat Diagn*. 2016;36(4):321-327. doi:10.1002/pd.4782

90. Jeng LB, Tarvin R, Robin NH. Genetic advances in central nervous system malformations in the fetus and neonate. *Semin Pediatr Neurol*. 2001;8(2):89-99. doi:10.1053/spen.2001.24836

91. Sun L, Wu Q, Jiang SW, Yan Y, Wang X, Zhang J, Liu Y, Yao L, Ma Y, Wang L. Prenatal Diagnosis of Central Nervous System Anomalies by High-Resolution Chromosomal Microarray Analysis. *BioMed Res Int*. 2015;2015:1-9. doi:10.1155/2015/426379

92. Krutzke SK, Engels H, Hofmann A, Schumann MM, Cremer K, Zink AM, Hilger A, Ludwig M, Gembruch U, Reutter H, Merz WM. Array-based molecular karyotyping in fetal brain malformations: Identification of novel candidate genes and chromosomal regions: CNV Analyses of Nonisolated CNS Malformations. *Birt Defects Res A Clin Mol Teratol*. 2016;106(1):16-26. doi:10.1002/bdra.23458

93. Schumann M, Hofmann A, Krutzke SK, Hilger AC, Marsch F, Stienen D, Gembruch U, Ludwig M, Merz WM, Reutter H. Array-based molecular karyotyping in fetuses with isolated brain malformations identifies disease-causing CNVs. *J Neurodev Disord*. 2016;8(1). doi:10.1186/s11689-016-9144-y

94. Cai M, Huang H, Xu L, Lin N. Clinical Utility and the Yield of Single Nucleotide Polymorphism Array in Prenatal Diagnosis of Fetal Central Nervous System Abnormalities. *Front Mol Biosci*. 2021;8:666115. doi:10.3389/fmolb.2021.666115

95. Scala C, Familiari A, Pinas A, Papageorghiou AT, Bhide A, Thilaganathan B, Khalil A. Perinatal and long-term outcomes in fetuses diagnosed with isolated unilateral ventriculomegaly: systematic review and meta-analysis: Outcomes in fetuses with

isolated unilateral ventriculomegaly. *Ultrasound Obstet Gynecol*. 2017;49(4):450-459. doi:10.1002/uog.15943

96. Signorelli M, Tiberti A, Valseriati D, Molin E, Cerri V, Groli C, Bianchi UA. Width of the fetal lateral ventricular atrium between 10 and 12 mm: a simple variation of the norm?: Variation in fetal lateral ventricular atrium width. *Ultrasound Obstet Gynecol*. 2004;23(1):14-18. doi:10.1002/uog.941

97. Kinzler WL, Smulian JC, McLean DA, Guzman ER, Vintzileos AM. Outcome of prenatally diagnosed mild unilateral cerebral ventriculomegaly. *J Ultrasound Med*. 2001;20(3):257-262. doi:10.7863/jum.2001.20.3.257

98. Melchiorre K, Bhide A, Gika AD, Pilu G, Papageorghiou AT. Counseling in isolated mild fetal ventriculomegaly. *Ultrasound Obstet Gynecol*. 2009;34(2):212-224. doi:10.1002/uog.7307

99. Barkovich AJ. *Congenital Malformations of the Brain and Skull.* 3rd ed. Pediatric Neuroimaging, Lippincott Williams & Wilkins; 2000.

100. Paladini D, Volpe P. *Ultrasound of Congenital Fetal Anomalies: Differential Diagnosis and Prognostic Indicators, Second Edition*. 0 ed. CRC Press; 2018. doi:10.4324/9780429462450

101. Dubourg C, Bendavid C, Pasquier L, Henry C, Odent S, David V. Holoprosencephaly. *Orphanet J Rare Dis*. 2007;2(1):8. doi:10.1186/1750-1172-2-8

102. Volpe P, Campobasso G, De Robertis V, Rembouskos G. Disorders of prosencephalic development. *Prenat Diagn*. 2009;29(4):340-354. doi:10.1002/pd.2208

103. Stevenson RE, ed. *Human Malformations and Related Anomalies*. 2nd ed. Oxford University Press; 2006.

104. Jeret JS, Serur D, Wisniewski K, Fisch C. Frequency of Agenesis of the Corpus Callosum in the Developmentally Disabled Population as Determined by Computerized Tomography. *Pediatr Neurosurg*. 1985;12(2):101-103. doi:10.1159/000120229

105. Palmer EE, Mowat D. Agenesis of the corpus callosum: A clinical approach to diagnosis: AMERICAN JOURNAL OF MEDICAL GENETICS PART C (SEMINARS IN MEDICAL GENETICS). *Am J Med Genet C Semin Med Genet*. 2014;166(2):184-197. doi:10.1002/ajmg.c.31405

106. DAntonio F, Pagani G, Familiari A, Khalil A, Sagies TL, Malinger G, Leibovitz Z, Garel C, Moutard ML, Pilu G, Bhide A, Acharya G, Leombroni M, Manzoli L, Papageorghiou A, Prefumo F. Outcomes Associated With Isolated Agenesis of the Corpus Callosum: A Meta-analysis. *PEDIATRICS*. 2016;138(3):e20160445-e20160445. doi:10.1542/peds.2016-0445

107. Bosemani T, Orman G, Boltshauser E, Tekes A, Huisman TAGM, Poretti A. Congenital Abnormalities of the Posterior Fossa. *RadioGraphics*. 2015;35(1):200-220. doi:10.1148/rg.351140038

108. Philip N. Mutations in the oligophrenin-1 gene (OPHN1) cause X linked congenital cerebellar hypoplasia. *J Med Genet*. 2003;40(6):441-446. doi:10.1136/jmg.40.6.441

109. D'Antonio F, Khalil A, Garel C, Pilu G, Rizzo G, Lerman-Sagie T, Bhide A, Thilaganathan B, Manzoli L, Papageorghiou AT. Systematic review and meta-analysis of isolated posterior fossa malformations on prenatal ultrasound imaging (part 1):

nomenclature, diagnostic accuracy and associated anomalies: Isolated posterior fossa malformations on prenatal imaging (part 1): meta-analysis. *Ultrasound Obstet Gynecol*. 2016;47(6):690-697. doi:10.1002/uog.14900

110. Lin HY, Lin SP, Chen YJ, Hung HY, Kao HA, Hsu CH, Chen MR, Chang JH, Ho CS, Huang FY, Shyur SD, Lin DS, Lee HC. Clinical characteristics and survival of trisomy 18 in a medical center in Taipei, 1988–2004. *Am J Med Genet A*. 2006;140A(9):945-951. doi:10.1002/ajmg.a.31173

111. Ulgiati F, Nicita F, Papetti L, Ursitti F, Di Maggio A, Tarani L, Spalice A. Posterior fossa malformations and sex chromosomes anomalies. Report of a case with XYY syndrome and overview of known associations. *Eur J Pediatr*. 2013;172(9):1267-1270. doi:10.1007/s00431-013-2039-y

112. Poretti A, Boltshauser E, Doherty D. Cerebellar hypoplasia: Differential diagnosis and diagnostic approach: AMERICAN JOURNAL OF MEDICAL GENETICS PART C (SEMINARS IN MEDICAL GENETICS). *Am J Med Genet C Semin Med Genet*. 2014;166(2):211-226. doi:10.1002/ajmg.c.31398

113. Sukhudyan B, Jaladyan V, Melikyan G, Schlump JU, Boltshauser E, Poretti A. Gómez–López-Hernández syndrome: reappraisal of the diagnostic criteria. *Eur J Pediatr*. 2010;169(12):1523-1528. doi:10.1007/s00431-010-1259-7

114. Ishak GE, Dempsey JC, Shaw DWW, Tully H, Adam MP, Sanchez-Lara PA, Glass I, Rue TC, Millen KJ, Dobyns WB, Doherty D. Rhombencephalosynapsis: a hindbrain malformation associated with incomplete separation of midbrain and forebrain,

hydrocephalus and a broad spectrum of severity. *Brain*. 2012;135(5):1370-1386. doi:10.1093/brain/aws065

115. Copp AJ, Adzick NS, Chitty LS, Fletcher JM, Holmbeck GN, Shaw GM. Spina bifida. *Nat Rev Dis Primer*. 2015;1:15007. doi:10.1038/nrdp.2015.7

116. Wallingford JB. Neural tube closure and neural tube defects: Studies in animal models reveal known knowns and known unknowns. *Am J Med Genet C Semin Med Genet*. 2005;135C(1):59-68. doi:10.1002/ajmg.c.30054

117. Greene NDE, Stanier P, Copp AJ. Genetics of human neural tube defects. *Hum Mol Genet*. 2009;18(R2):R113-R129. doi:10.1093/hmg/ddp347

118. Busby A, Dolk H, Collin R, Jones RB, Winter R. Compiling a national register of babies born with anophthalmia/microphthalmia in England 1988-94. *Arch Dis Child - Fetal Neonatal Ed*. 1998;79(3):F168-F173. doi:10.1136/fn.79.3.F168

119. Benacerraf BR. *Ultrasound of Fetal Syndromes*. 2. ed. Elsevier, Churchill Livingstone; 2008.

120. Pineda-Alvarez DE, Solomon BD, Roessler E, Balog JZ, Hadley DW, Zein WM, Hadsall CK, Brooks BP, Muenke M. A broad range of ophthalmologic anomalies is part of the holoprosencephaly spectrum. *Am J Med Genet A*. 2011;155(11):2713-2720. doi:10.1002/ajmg.a.34261

121. Ondeck CL, Pretorius D, McCaulley J, Kinori M, Maloney T, Hull A, Robbins SL. Ultrasonographic prenatal imaging of fetal ocular and orbital abnormalities. *Surv Ophthalmol*. 2018;63(6):745-753. doi:10.1016/j.survophthal.2018.04.006

122. Dixon MJ, Marazita ML, Beaty TH, Murray JC. Cleft lip and palate: understanding genetic and environmental influences. *Nat Rev Genet*. 2011;12(3):167-178. doi:10.1038/nrg2933

123. Offerdal K, Jebens N, Syvertsen T, Blaas HGK, Johansen OJ, Eik-Nes SH. Prenatal ultrasound detection of facial clefts: a prospective study of 49 314 deliveries in a non-selected population in Norway. *Ultrasound Obstet Gynecol*. 2008;31(6):639-646. doi:10.1002/uog.5280

124. de Boutray M, Beziat JL, Yachouh J, Bigorre M, Gleizal A, Captier G. Median cleft of the upper lip: A new classification to guide treatment decisions. *J Cranio-Maxillofac Surg*. 2016;44(6):664-671. doi:10.1016/j.jcms.2016.02.012

125. Martinelli P, Maruotti GM, Agangi A, Mazzarelli LL, Bifulco G, Paladini D. Prenatal diagnosis of hemifacial microsomia and ipsilateral cerebellar hypoplasia in a fetus with oculoauriculovertebral spectrum: OAVS and cerebellar hemihypoplasia. *Ultrasound Obstet Gynecol*. 2004;24(2):199-201. doi:10.1002/uog.1118

126. Maruotti GM, Paladini D, Agangi A, Martinelli P. Prospective prenatal diagnosis of Fraser syndrome variant in a family with negative history: LETTERS TO THE EDITOR. *Prenat Diagn*. 2004;24(1):69-70. doi:10.1002/pd.769

127. Koul M, Dwivedi R, Upadhyay V. Ectrodactyly-ectodermal dysplasia clefting syndrome (EEC syndrome). *J Oral Biol Craniofacial Res*. 2014;4(2):135-139. doi:10.1016/j.jobcr.2014.08.002

128. Vettraino I. Clinical outcome of fetuses with sonographic diagnosis of isolated micrognathia. *Obstet Gynecol*. 2003;102(4):801-805. doi:10.1016/S0029-7844(03)00672-0

129. Paladini D. Fetal micrognathia: almost always an ominous finding. *Ultrasound Obstet Gynecol*. 2010;35(4):377-384. doi:10.1002/uog.7639

130. Andrade CF, Ferreira HP da C, Fischer GB. Malformações pulmonares congênitas. *J Bras Pneumol*. 2011;37(2):259-271. doi:10.1590/S1806-37132011000200017

131. Dolk H, Loane M, Garne E. The Prevalence of Congenital Anomalies in Europe. In: Posada de la Paz M, Groft SC, eds. *Rare Diseases Epidemiology*. Vol 686. Advances in Experimental Medicine and Biology. Springer Netherlands; 2010:349-364. doi:10.1007/978-90-481-9485-8_20

132. Paladini D, Borghese A, Arienzo M, Teodoro A, Martinelli P, Nappi C. Prospective ultrasound diagnosis of Pallister-Killian syndrome in the second trimester of pregnancy: the importance of the fetal facial profile. *Prenat Diagn*. 2000;20(12):996-998.

133. McPherson EW, Ketterer DM, Salsburey DJ. Pallister-Killian and Fryns syndromes: Nosology. *Am J Med Genet*. 1993;47(2):241-245. doi:10.1002/ajmg.1320470219

134. Kammoun M, Souche E, Brady P, Ding J, Cosemans N, Gratacos E, Devriendt K, Eixarch E, Deprest J, Vermeesch JR. Genetic profile of isolated congenital diaphragmatic hernia revealed by targeted next-generation sequencing. *Prenat Diagn*. 2018;38(9):654-663. doi:10.1002/pd.5327

135. Pober B. Genetic aspects of human congenital diaphragmatic hernia. *Clin Genet*. 2008;74(1):1-15. doi:10.1111/j.1399-0004.2008.01031.x

136. Gezer S, Taştepe İ, Sırmalı M, Fındık G, Türüt H, Kaya S, Karaoğlanoğlu N, Çetin G. Pulmonary sequestration: A single-institutional series composed of 27 cases. *J Thorac Cardiovasc Surg*. 2007;133(4):955-959. doi:10.1016/j.jtcvs.2006.11.003

137. De Bernardo G, Giordano M, Di Toro A, Sordino D, De Brasi D. Prenatal diagnosis of Fraser syndrome: a matter of life or death? *Ital J Pediatr*. 2015;41(1):86. doi:10.1186/s13052-015-0195-6

138. Longaker MT, Laberge JM, Dansereau J, Langer JC, Crombleholme TM, Callen PW, Golbus MS, Harrison MR. Primary fetal hydrothorax: Natural history and management. *J Pediatr Surg*. 1989;24(6):573-576. doi:10.1016/S0022-3468(89)80509-3

139. Achiron R, Weissman A, Lipitz S, Mashiach S, Goldman B. Fetal Pleural Effusion: The Risk of Fetal Trisomy. *Gynecol Obstet Invest*. 1995;39(3):153-156. doi:10.1159/000292399

140. Aubard Y, Derouineau I, Aubard V, Chalifour V, Preux PM. Primary Fetal Hydrothorax: A Literature Review and Proposed Antenatal Clinical Strategy. *Fetal Diagn Ther*. 1998;13(6):325-333. doi:10.1159/000020863

141. Lupo PJ, Isenburg JL, Salemi JL, Mai CT, Liberman RF, Canfield MA, Copeland G, Haight S, Harpavat S, Hoyt AT, Moore CA, Nembhard WN, Nguyen HN, Rutkowski RE, Steele A, Alverson CJ, Stallings EB, Kirby RS, and The National Birth Defects Prevention Network. Population-based birth defects data in the United States, 2010-2014: A focus

on gastrointestinal defects. *Birth Defects Res*. 2017;109(18):1504-1514. doi:10.1002/bdr2.1145

142. Solomon BD, Bear KA, Kimonis V, de Klein A, Scott DA, Shaw-Smith C, Tibboel D, Reutter H, Giampietro PF. Clinical geneticists' views of VACTERL/VATER association. *Am J Med Genet A*. 2012;158A(12):3087-3100. doi:10.1002/ajmg.a.35638

143. de Jong EM, de Haan MAM, Gischler SJ, Hop W, Cohen-Overbeek TE, Bax NMA, de Klein A, Tibboel D, Grijseels EWM. Pre- and postnatal diagnosis and outcome of fetuses and neonates with esophageal atresia and tracheoesophageal fistula: NEONATES WITH ESOPHAGEAL ATRESIA AND TRACHEOESOPHAGEAL FISTULA. *Prenat Diagn*. 2010;30(3):274-279. doi:10.1002/pd.2466

144. Escobar MA, Ladd AP, Grosfeld JL, West KW, Rescorla FJ, Scherer LR, Engum SA, Rouse TM, Billmire DF. Duodenal atresia and stenosis: long-term follow-up over 30 years. *J Pediatr Surg*. 2004;39(6):867-871. doi:10.1016/j.jpedsurg.2004.02.025

145. Choudhry MS, Rahman N, Boyd P, Lakhoo K. Duodenal atresia: associated anomalies, prenatal diagnosis and outcome. *Pediatr Surg Int*. 2009;25(8):727-730. doi:10.1007/s00383-009-2406-y

146. Bishop JC, McCormick B, Johnson CT, Miller J, Jelin E, Blakemore K, Jelin AC. The Double Bubble Sign: Duodenal Atresia and Associated Genetic Etiologies. *Fetal Diagn Ther*. 2020;47(2):98-103. doi:10.1159/000500471

147. Hou JW, Wang TR. Amelia, dextrocardia, asplenia, and congenital short bowel in deleted ring chromosome 4. *J Med Genet*. 1996;33(10):879-881. doi:10.1136/jmg.33.10.879

148. Barisic I, Clementi M, Häusler M, Gjergja R, Kern J, Stoll C. Evaluation of prenatal ultrasound diagnosis of fetal abdominal wall defects by 19 European registries: Abdominal wall defects. *Ultrasound Obstet Gynecol*. 2001;18(4):309-316. doi:10.1046/j.0960-7692.2001.00534.x

149. Lakasing L, Cicero S, Davenport M, Patel S, Nicolaides KH. Current outcome of antenatally diagnosed exomphalos: an 11 year review. *J Pediatr Surg*. 2006;41(8):1403-1406. doi:10.1016/j.jpedsurg.2006.04.015

150. Rankin J, Dillon E, Wright C. Congenital anterior abdominal wall defects in the north of England, 1986-1996: occurrence and outcome. *Prenat Diagn*. 1999;19(7):662-668. doi:10.1002/(sici)1097-0223(199907)19:7<662::aid-pd607>3.0.co;2-c

151. St-Vil D, Shaw KS, Lallier M, Yazbeck S, Di Lorenzo M, Grignon A, Blanchard H. Chromosomal anomalies in newborns with omphalocele. *J Pediatr Surg*. 1996;31(6):831-834. doi:10.1016/S0022-3468(96)90146-3

152. Kirby RS, Marshall J, Tanner JP, Salemi JL, Feldkamp ML, Marengo L, Meyer RE, Druschel CM, Rickard R, Kucik JE. Prevalence and Correlates of Gastroschisis in 15 States, 1995 to 2005. *Obstet Gynecol*. 2013;122(2):275-281. doi:10.1097/AOG.0b013e31829cbbb4

153. Mastroiacovo P, Lisi A, Castilla EE, Martínez-Frías ML, Bermejo E, Marengo L, Kucik J, Siffel C, Halliday J, Gatt M, Annerèn G, Bianchi F, Canessa MA, Danderfer R, de Walle H, Harris J, Li Z, Lowry RB, McDonell R, Merlob P, Metneki J, Mutchinick O, Robert-Gnansia E, Scarano G, Sipek A, Pötzsch S, Szabova E, Yevtushok L. Gastroschisis and

associated defects: An international study. *Am J Med Genet A*. 2007;143A(7):660-671. doi:10.1002/ajmg.a.31607

154. Jenetzky E. Prevalence estimation of anorectal malformations using German diagnosis related groups system. *Pediatr Surg Int*. 2007;23(12):1161-1165. doi:10.1007/s00383-007-2023-6

155. Zwink N, Jenetzky E, Brenner H. Parental risk factors and anorectal malformations: systematic review and meta-analysis. *Orphanet J Rare Dis*. 2011;6(1):25. doi:10.1186/1750-1172-6-25

156. Dworschak GC, Draaken M, Marcelis C, de Blaauw I, Pfundt R, van Rooij IALM, Bartels E, Hilger A, Jenetzky E, Schmiedeke E, Grasshoff-Derr S, Schmidt D, Märzheuser S, Hosie S, Weih S, Holland-Cunz S, Palta M, Leonhardt J, Schäfer M, Kujath C, Rißmann A, Nöthen MM, Zwink N, Ludwig M, Reutter H. De novo 13q deletions in two patients with mild anorectal malformations as part of VATER/VACTERL and VATER/VACTERL-like association and analysis of *EFNB2* in patients with anorectal malformations. *Am J Med Genet A*. 2013;161(12):3035-3041. doi:10.1002/ajmg.a.36153

157. Schramm C, Draaken M, Bartels E, Boemers TM, Aretz S, Brockschmidt FF, Nöthen MM, Ludwig M, Reutter H. De novo microduplication at 22q11.21 in a patient with VACTERL association. *Eur J Med Genet*. 2011;54(1):9-13. doi:10.1016/j.ejmg.2010.09.001

158. Grandjean H, Larroque D, Levi S. The performance of routine ultrasonographic screening of pregnancies in the Eurofetus Study. *Am J Obstet Gynecol*. 1999;181(2):446-454. doi:10.1016/S0002-9378(99)70577-6

159. Sanna-Cherchi S, Westland R, Ghiggeri GM, Gharavi AG. Genetic basis of human congenital anomalies of the kidney and urinary tract. *J Clin Invest*. 2018;128(1):4-15. doi:10.1172/JCI95300

160. Gruskin D, Kanil E, Rimoin D. Congenital disorders of the urinary tract. In: Rimoin D, Conor JM, Pyeritz RE, Korf BR, eds. Emery and Rimoin's principles and practice of medical Genetics , 4TH edn.Edinburgh. In: *Emery and Rimoin's Principles and Practice of Medical Genetics ,*. 4TH edn. Churchill-Livingstone; 2002:1659-1743.

161. Stonebrook E, Hoff M, Spencer JD. Congenital Anomalies of the Kidney and Urinary Tract: a Clinical Review. *Curr Treat Options Pediatr*. 2019;5(3):223-235. doi:10.1007/s40746-019-00166-3

162. Yulia A, Winyard P. Management of antenatally detected kidney malformations. *Early Hum Dev*. 2018;126:38-46. doi:10.1016/j.earlhumdev.2018.08.017

163. Feldenberg LR, Siegel NJ. Clinical course and outcome for children with multicystic dysplastic kidneys. *Pediatr Nephrol*. 2000;14(12):1098-1101. doi:10.1007/s004670000391

164. Rudnik-Schöneborn S, John U, Deget F, Ehrich JHH, Misselwitz J, Zerres K. Clinical features of unilateral multicystic renal dysplasia in children. *Eur J Pediatr*. 1998;157(8):666-672. doi:10.1007/s004310050908

165. Chen RY, Chang H. Renal Dysplasia. *Arch Pathol Lab Med*. 2015;139(4):547-551. doi:10.5858/arpa.2013-0660-RS

166. Mori M, Matsubara K, Abe E, Matsubara Y, Katayama T, Fujioka T, Kusanagi Y, Ito M. Prenatal Diagnosis of Persistent Cloaca Associated with VATER (Vertebral Defects, Anal Atresia, Tracheo-Esophageal Fistula, and Renal Dysplasia). *Tohoku J Exp Med*. 2007;213(4):291-295. doi:10.1620/tjem.213.291

167. Mouriquand PD, Whitten M, Pracros JP. Pathophysiology, diagnosis and management of prenatal upper tract dilatation. *Prenat Diagn*. 2001;21(11):942-951.

168. Fontanella F, Maggio L, Verheij JBGM, Duin LK, Adama Van Scheltema PN, Cohen-Overbeek TE, Pajkrt E, Bekker M, Willekes C, Bax CJ, Gracchi V, Oepkes D, Bilardo CM. Fetal megacystis: a lot more than LUTO. *Ultrasound Obstet Gynecol*. 2019;53(6):779-787. doi:10.1002/uog.19182

169. Richardson EJ, Scott FP, McLennan AC. Sex discordance identification following non-invasive prenatal testing. *Prenat Diagn*. 2017;37(13):1298-1304. doi:10.1002/pd.5184

170. Cheikhelard A, Luton D, Philippe-Chomette P, Leger J, Vuillard E, Garel C, Polak M, Nessmann C, Aigrain Y, El-GHONEIMI A. How accurate is the prenatal diagnosis of abnormal genitalia? *J Urol*. Published online September 2000:984-987. doi:10.1097/00005392-200009020-00016

171. Mortier GR, Cohn DH, Cormier-Daire V, Hall C, Krakow D, Mundlos S, Nishimura G, Robertson S, Sangiorgi L, Savarirayan R, Sillence D, Superti-Furga A, Unger S, Warman ML. Nosology and classification of genetic skeletal disorders: 2019 revision. *Am J Med Genet A*. 2019;179(12):2393-2419. doi:10.1002/ajmg.a.61366

172. Superti-Furga A, Unger S, and the Nosology Group of the International Skeletal Dysplasia Society. Nosology and classification of genetic skeletal disorders: 2006 revision. *Am J Med Genet A*. 2007;143A(1):1-18. doi:10.1002/ajmg.a.31483

173. Shanske AL, Bernstein L, Herzog R. Chondrodysplasia Punctata and Maternal Autoimmune Disease: A New Case and Review of the Literature. *PEDIATRICS*. 2007;120(2):e436-e441. doi:10.1542/peds.2006-2997

174. Siegrist Ridruejo J, Bravo Arribas C, Antolín Alvarado E, de León Luis JA, Gámez Aldarete F, Pérez Fernández-Pacheco R. Malformaciones esqueléticas: diagnóstico ecográfico y resultados perinatales. *Diagnóstico Prenat*. 2011;22(1):7-13. doi:10.1016/j.diapre.2010.01.005

175. Malik S. Polydactyly: phenotypes, genetics and classification. *Clin Genet*. 2014;85(3):203-212. doi:10.1111/cge.12276

176. Barisic I, Boban L, Loane M, Garne E, Wellesley D, Calzolari E, Dolk H, Addor MC, Bergman JE, Braz P, Draper ES, Haeusler M, Khoshnood B, Klungsoyr K, Pierini A, Queisser-Luft A, Rankin J, Rissmann A, Verellen-Dumoulin C. Meckel–Gruber Syndrome: a population-based study on prevalence, prenatal diagnosis, clinical features, and survival in Europe. *Eur J Hum Genet*. 2015;23(6):746-752. doi:10.1038/ejhg.2014.174

177. DeBarber AE, Eroglu Y, Merkens LS, Pappu AS, Steiner RD. Smith–Lemli–Opitz syndrome. *Expert Rev Mol Med*. 2011;13:e24. doi:10.1017/S146239941100189X

178. Chandra S, Daryappa M, Mukheem Mudabbir M, Pooja M, Arivazhagan A. Pallister–Hall syndrome. *J Pediatr Neurosci*. 2017;12(3):276. doi:10.4103/jpn.JPN_101_17

179. Biesecker LG. The Greig cephalopolysyndactyly syndrome. *Orphanet J Rare Dis*. 2008;3(1):10. doi:10.1186/1750-1172-3-10

180. Malik S, Ullah S, Afzal M, Lal K, Haque S. Clinical and descriptive genetic study of polydactyly: a Pakistani experience of 313 cases: Descriptive genetic study of polydactyly in Pakistan. *Clin Genet*. 2014;85(5):482-486. doi:10.1111/cge.12217

181. Werler MM, Yazdy MM, Mitchell AA, Meyer RE, Druschel CM, Anderka M, Kasser JR, Mahan ST. Descriptive epidemiology of idiopathic clubfoot. *Am J Med Genet A*. 2013;161(7):1569-1578. doi:10.1002/ajmg.a.35955

182. Viaris de le Segno B, Gruchy N, Bronfen C, Dolley P, Leporrier N, Creveuil C, Benoist G. Prenatal diagnosis of clubfoot: Chromosomal abnormalities associated with fetal defects and outcome in a tertiary center: Prenatal Diagnosis of Clubfoot. *J Clin Ultrasound*. 2016;44(2):100-105. doi:10.1002/jcu.22275

183. Cheung KW, Lai CWS, Mak CCY, Hui PW, Chung BHY, Kan ASY. A case of prenatal isolated talipes and 22q11.2 deletion syndrome-an important chromosomal disorder missed by noninvasive prenatal screening. *Prenat Diagn*. 2018;38(5):376-378. doi:10.1002/pd.5241

184. McKinney J, Rac MWF, Gandhi M. Congenital talipes equinovarus (clubfoot). *Am J Obstet Gynecol*. 2019;221(6):B10-B12. doi:10.1016/j.ajog.2019.09.022

185. Norton ME, Chauhan SP, Dashe JS. Society for Maternal-Fetal Medicine (SMFM) Clinical Guideline #7: nonimmune hydrops fetalis. *Am J Obstet Gynecol*. 2015;212(2):127-139. doi:10.1016/j.ajog.2014.12.018

186. Santolaya J, Alley D, Jaffe R, Warsof SL. Antenatal classification of hydrops fetalis. *Obstet Gynecol*. 1992;79(2):256-259.

187. Sparks TN, Thao K, Lianoglou BR, Boe NM, Bruce KG, Datkhaeva I, Field NT, Fratto VM, Jolley J, Laurent LC, Mardy AH, Murphy AM, Ngan E, Rangwala N, Rottkamp CAM, Wilson L, Wu E, Uy CC, Valdez Lopez P, Norton ME. Nonimmune hydrops fetalis: identifying the underlying genetic etiology. *Genet Med*. 2019;21(6):1339-1344. doi:10.1038/s41436-018-0352-6

188. Bellini C, Hennekam RCM, Fulcheri E, Rutigliani M, Morcaldi G, Boccardo F, Bonioli E. Etiology of nonimmune hydrops fetalis: A systematic review. *Am J Med Genet A*. 2009;149A(5):844-851. doi:10.1002/ajmg.a.32655

189. Acar A, Balci O, Gezginc K, Onder C, Capar M, Zamani A, Acar A. Evaluation of the Results of Cordocentesis. *Taiwan J Obstet Gynecol*. 2007;46(4):405-409. doi:10.1016/S1028-4559(08)60011-X

190. Gimovsky AC, Luzi P, Berghella V. Lysosomal storage disease as an etiology of nonimmune hydrops. *Am J Obstet Gynecol*. 2015;212(3):281-290. doi:10.1016/j.ajog.2014.10.007

191. Sonographic examination of the fetal central nervous system: guidelines for performing the 'basic examination' and the 'fetal neurosonogram.' *Ultrasound Obstet Gynecol*. 2007;29(1):109-116. doi:10.1002/uog.3909

192. Carvalho J, Allan L, Chaoui R, Copel J, DeVore G, Hecher K, Lee W, Munoz H, Paladini D, Tutschek B, Yagel S. ISUOG Practice Guidelines (updated): sonographic

screening examination of the fetal heart: ISUOG Guidelines. *Ultrasound Obstet Gynecol*. 2013;41(3):348-359. doi:10.1002/uog.12403

193. ClinGen Genome Dosage Map. Accessed August 30, 2020. https://dosage.clinicalgenome.org/

194. Hillman SC, Pretlove S, Coomarasamy A, Mcmullan DJ, Davison EV, Maher ER, Kilby MD. Additional information from array comparative genomic hybridization technology over conventional karyotyping in prenatal diagnosis: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2011;37(1):6-14. doi:10.1002/uog.7754

195. Riggs ER, Andersen EF, Cherry AM, Kantarci S, Kearney H, Patel A, Raca G, Ritter DI, South ST, Thorland EC, Pineda-Alvarez D, Aradhya S, Martin CL. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). *Genet Med*. 2020;22(2):245-257. doi:10.1038/s41436-019-0686-8

196. Muys J, Blaumeiser B, Jacquemyn Y, Bandelier C, Brison N, Bulk S, Chiarappa P, Courtens W, De Leener A, De Rademaeker M, Désir J, Destrée A, Devriendt K, Dheedene A, Fieuw A, Fransen E, Gatot J, Holmgren P, Jamar M, Janssens S, Keymolen K, Lederer D, Menten B, Meuwissen M, Parmentier B, Pichon B, Rombout S, Sznajer Y, Van Den Bogaert A, Van Den Bogaert K, Vanakker O, Vermeesch J, Janssens K. The Belgian MicroArray Prenatal (BEMAPRE) database: A systematic nationwide repository of fetal genomic aberrations. *Prenat Diagn*. 2018;38(13):1120-1128. doi:10.1002/pd.5373

197. Vanakker O, Vilain C, Janssens K, Van der Aa N, Smits G, Bandelier C, Blaumeiser B, Bulk S, Caberg JH, De Leener A, De Rademaeker M, de Ravel T, Desir J, Destree A, Dheedene A, Gaillez S, Grisart B, Hellin AC, Janssens S, Keymolen K, Menten B, Pichon B, Ravoet M, Revencu N, Rombout S, Staessens C, Van Den Bogaert A, Van Den Bogaert K, Vermeesch JR, Kooy F, Sznajer Y, Devriendt K. Implementation of genomic arrays in prenatal diagnosis: The Belgian approach to meet the challenges. *Eur J Med Genet*. 2014;57(4):151-156. doi:10.1016/j.ejmg.2014.02.002

198. Shaffer LG, Rosenfeld JA, Dabell MP, Coppinger J, Bandholz AM, Ellison JW, Ravnan JB, Torchia BS, Ballif BC, Fisher AJ. Detection rates of clinically significant genomic alterations by microarray analysis for specific anomalies detected by ultrasound: Microarray experience with abnormal ultrasound anomalies. *Prenat Diagn*. 2012;32(10):986-995. doi:10.1002/pd.3943

199. The Royal of Pathologists, BSGM, Royal College of Obstetricians & Gynaecologists. Recommendations for the use of chromosome microarray in pregnancy. Published June 2015. Accessed August 30, 2020. https://www.rcpath.org/uploads/assets/06664c28-0f90-4230-86158c91fea14be6/Recommendations-for-the-use-of-chromosome-microarray-in-pregnancy.pdf

200. Gordijn SJ, Beune IM, Thilaganathan B, Papageorghiou A, Baschat AA, Baker PN, Silver RM, Wynia K, Ganzevoort W. Consensus definition of fetal growth restriction: a Delphi procedure: Consensus definition of FGR. *Ultrasound Obstet Gynecol*. 2016;48(3):333-339. doi:10.1002/uog.15884

201. Nguyen HT, Benson CB, Bromley B, Campbell JB, Chow J, Coleman B, Cooper C, Crino J, Darge K, Anthony Herndon CD, Odibo AO, Somers MJG, Stein DR. Multidisciplinary consensus on the classification of prenatal and postnatal urinary tract dilation (UTD classification system). *J Pediatr Urol*. 2014;10(6):982-998. doi:10.1016/j.jpurol.2014.10.002

202. Santirocco M, Plaja A, Rodó C, Valenzuela I, Arévalo S, Castells N, Abuli A, Tizzano E, Maiz N, Carreras E. Chromosomal microarray analysis in fetuses with central nervous system anomalies: An 8-year long observational study from a tertiary care university hospital. *Prenat Diagn*. Published online September 30, 2020:pd.5829. doi:10.1002/pd.5829

203. Chen CP, Ko TM, Huang WC, Chern SR, Wu PS, Chen YN, Chen SW, Lee CC, Pan CW, Yang CW, Wang W. Molecular cytogenetic characterization of inv dup del(8p) in a fetus associated with ventriculomegaly, hypoplastic left heart, polyhydramnios and intestinal obstruction. *Taiwan J Obstet Gynecol*. 2016;55(3):415-418. doi:10.1016/j.tjog.2016.05.001

204. Verhagen JMA, de Leeuw N, Papatsonis DNM, Grijseels EWM, de Krijger RR, Wessels MW. Phenotypic Variability Associated with a Large Recurrent 1q21.1 Microduplication in a Three-Generation Family. *Mol Syndromol*. 2015;6(2):71-76. doi:10.1159/000431274

205. O'Driscoll MC, Black GCM, Clayton-Smith J, Sherr EH, Dobyns WB. Identification of genomic loci contributing to agenesis of the corpus callosum. *Am J Med Genet A*. 2010;152A(9):2145-2159. doi:10.1002/ajmg.a.33558

206. Peddibhotla S, Nagamani SC, Erez A, Hunter JV, Holder Jr JL, Carlin ME, Bader PI, Perras HM, Allanson JE, Newman L, Simpson G, Immken L, Powell E, Mohanty A, Kang SHL, Stankiewicz P, Bacino CA, Bi W, Patel A, Cheung SW. Delineation of candidate genes responsible for structural brain abnormalities in patients with terminal deletions of chromosome 6q27. *Eur J Hum Genet*. 2015;23(1):54-60. doi:10.1038/ejhg.2014.51

207. Mercier S, Dubourg C, Garcelon N, Campillo-Gimenez B, Gicquel I, Belleguic M, Ratie L, Pasquier L, Loget P, Bendavid C, Jaillard S, Rochard L, Quelin C, Dupe V, David V, Odent S. New findings for phenotype-genotype correlations in a large European series of holoprosencephaly cases. *J Med Genet*. 2011;48(11):752-760. doi:10.1136/jmedgenet-2011-100339

208. Solomon BD, Lacbawan F, Mercier S, Clegg NJ, Delgado MR, Rosenbaum K, Dubourg C, David V, Olney AH, Wehner LE, Hehr U, Bale S, Paulussen A, Smeets HJ, Hardisty E, Tylki-Szymanska A, Pronicka E, Clemens M, McPherson E, Hennekam RCM, Hahn J, Stashinko E, Levey E, Wieczorek D, Roeder E, Schell-Apacik CC, Booth CW, Thomas RL, Kenwrick S, Cummings DAT, Bous SM, Keaton A, Balog JZ, Hadley D, Zhou N, Long R, Velez JI, Pineda-Alvarez DE, Odent S, Roessler E, Muenke M. Mutations in ZIC2 in human holoprosencephaly: description of a Novel ZIC2 specific phenotype and comprehensive analysis of 157 individuals. *J Med Genet*. 2010;47(8):513-524. doi:10.1136/jmg.2009.073049

209. Scott D, Jordan V, Zaveri H. 1p36 deletion syndrome: an update. *Appl Clin Genet*. Published online August 2015:189. doi:10.2147/TACG.S65698

210. Kor-anantakul O, Suwanrath C, Kanngurn S, Rujirabanjerd S, Suntharasaj T, Pinjaroen S. Prenatal Diagnosis of Complete Trisomy 9: A Case Report and Review of the Literature. *Am J Perinatol*. 2006;23(02):131-136. doi:10.1055/s-2006-931804

211. Uguen A, Talagas M, Quémener-Redon S, Marcorelles P, De Braekeleer M. Duplication of SOX3 (Xq27) may be a risk factor for Neural Tube Defects. *Am J Med Genet A*. 2015;167(7):1676-1678. doi:10.1002/ajmg.a.37072

212. Hureaux M, Ben Miled S, Chatron N, Coussement A, Bessières B, Egloff M, Mechler C, Stirnemann J, Tsatsaris V, Barcia G, Turleau C, Ville Y, Encha-Razavi F, Attie-Bitach T, Malan V. SOX3 duplication: a genetic cause to investigate in fetuses with neural tube defects. *Prenat Diagn*. 2019;39(11):1026-1034. doi:10.1002/pd.5523

213. Yu HC, Coughlin CR, Geiger EA, Salvador BJ, Elias ER, Cavanaugh JL, Chatfield KC, Miyamoto SD, Shaikh TH. Discovery of a potentially deleterious variant in *TMEM87B* in a patient with a hemizygous 2q13 microdeletion suggests a recessive condition characterized by congenital heart disease and restrictive cardiomyopathy. *Mol Case Stud*. 2016;2(3):a000844. doi:10.1101/mcs.a000844

214. Riley KN, Catalano LM, Bernat JA, Adams SD, Martin DM, Lalani SR, Patel A, Burnside RD, Innis JW, Rudd MK. Recurrent deletions and duplications of chromosome 2q11.2 and 2q13 are associated with variable outcomes. *Am J Med Genet A*. 2015;167(11):2664-2673. doi:10.1002/ajmg.a.37269

215. Huynh MT, Lambert AS, Tosca L, Petit F, Philippe C, Parisot F, Benoît V, Linglart A, Brisset S, Tran CT, Tachdjian G, Receveur A. 15q24.1 BP4-BP1 microdeletion unmasking paternally inherited functional polymorphisms combined with distal

15q24.2q24.3 duplication in a patient with epilepsy, psychomotor delay, overweight, ventricular arrhythmia. *Eur J Med Genet*. 2018;61(8):459-464. doi:10.1016/j.ejmg.2018.03.005

216. Samuelsson L, Zagoras T, Hafström M. Inherited 15q24 microdeletion syndrome in twins and their father with phenotypic variability. *Eur J Med Genet*. 2015;58(2):111-115. doi:10.1016/j.ejmg.2014.12.006

217. Onesimo R, Orteschi D, Scalzone M, Rossodivita A, Nanni L, Zannoni GF, Marrocco G, Battaglia D, Fundarò C, Neri G. Chromosome 9p deletion syndrome and sex reversal: Novel findings and redefinition of the critically deleted regions. *Am J Med Genet A*. 2012;158A(9):2266-2271. doi:10.1002/ajmg.a.35489

218. Scholz C, Steinemann D, Mälzer M, Roy M, Arslan-Kirchner M, Illig T, Schmidtke J, Stuhrmann M. NCAM2 deletion in a boy with macrocephaly and autism: Cause, association or predisposition? *Eur J Med Genet*. 2016;59(10):493-498. doi:10.1016/j.ejmg.2016.08.006

219. Çetin ÖE, Yalçınkaya C, Karaman B, Demirbilek V, Tüysüz B. Chromosome 14q11.2-q21.1 duplication: a rare cause of West syndrome. *Epileptic Disord*. 2018;20(3):219-224. doi:10.1684/epd.2018.0972

220. Amor DJ, Burgess T, Tan TY, Pertile MD. Questionable pathogenicity of FOXG1 duplication. *Eur J Hum Genet*. 2012;20(6):595-596. doi:10.1038/ejhg.2011.267

221. Keeling SL, Lee-Jones L, Thompson P. Interstitial deletion 4q32-34 with ulnar deficiency: 4q33 may be the critical region in 4q terminal deletion syndrome. *Am J Med*

Genet. 2001;99(2):94-98. doi:10.1002/1096-8628(2000)9999:999<00::AID-AJMG1134>3.0.CO;2-D

222. Pinto IP, Minasi LB, Steckelberg R, da Silva CC, da Cruz AD. Mosaic Tetrasomy of 9p24.3q21.11 postnatally identified in an infant born with multiple congenital malformations: a case report. *BMC Pediatr*. 2018;18(1):298. doi:10.1186/s12887-018-1275-8

223. Hobart HH, Morris CA, Mervis CB, Pani AM, Kistler DJ, Rios CM, Kimberley KW, Gregg RG, Bray-Ward P. Inversion of the Williams syndrome region is a common polymorphism found more frequently in parents of children with Williams syndrome. *Am J Med Genet C Semin Med Genet*. 2010;154C(2):220-228. doi:10.1002/ajmg.c.30258

224. D'Amours G, Kibar Z, Mathonnet G, Fetni R, Tihy F, Désilets V, Nizard S, Michaud J, Lemyre E. Whole-genome array CGH identifies pathogenic copy number variations in fetuses with major malformations and a normal karyotype. *Clin Genet*. 2012;81(2):128-141. doi:10.1111/j.1399-0004.2011.01687.x

225. Lovrecic L, Remec ZI, Volk M, Rudolf G, Writzl K, Peterlin B. Clinical utility of array comparative genomic hybridisation in prenatal setting. *BMC Med Genet*. 2016;17(1):81. doi:10.1186/s12881-016-0345-8

226. Srebniak MI, Boter M, Oudesluijs GO, Cohen-Overbeek T, Govaerts LC, Diderich KE, Oegema R, Knapen MF, van de Laar IM, Joosten M, Van Opstal D, Galjaard RJH. Genomic SNP array as a gold standard for prenatal diagnosis of foetal ultrasound abnormalities. *Mol Cytogenet*. 2012;5(1):14. doi:10.1186/1755-8166-5-14

227. Sun L, Wu Q, Jiang SW, Yan Y, Wang X, Zhang J, Liu Y, Yao L, Ma Y, Wang L. Prenatal Diagnosis of Central Nervous System Anomalies by High-Resolution Chromosomal Microarray Analysis. *BioMed Res Int*. 2015;2015:426379 (9 pages). doi:10.1155/2015/426379

228. Schumann M, Hofmann A, Krutzke SK, Hilger AC, Marsch F, Stienen D, Gembruch U, Ludwig M, Merz WM, Reutter H. Array-based molecular karyotyping in fetuses with isolated brain malformations identifies disease-causing CNVs. *J Neurodev Disord*. 2016;8(1):11. doi:10.1186/s11689-016-9144-y

229. Krutzke SK, Engels H, Hofmann A, Schumann MM, Cremer K, Zink AM, Hilger A, Ludwig M, Gembruch U, Reutter H, Merz WM. Array-based molecular karyotyping in fetal brain malformations: Identification of novel candidate genes and chromosomal regions: CNV Analyses of Nonisolated CNS Malformations. *Birt Defects Res A Clin Mol Teratol*. 2016;106(1):16-26. doi:10.1002/bdra.23458

230. Li Z, Fu F, Lei T, Li R, Jing X, Yang X, Han J, Pan M, Zhen L, Liao C. [Application of chromosome microarray analysis for the delineation of pathogenesis for fetal ventriculomegaly]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi Zhonghua Yixue Yichuanxue Zazhi Chin J Med Genet*. 2017;34(4):576-582. doi:10.3760/cma.j.issn.1003-9406.2017.04.024

231. Di Gregorio E, Savin E, Biamino E, Belligni EF, Naretto VG, D'Alessandro G, Gai G, Fiocchi F, Calcia A, Mancini C, Giorgio E, Cavalieri S, Talarico F, Pappi P, Gandione M, Grosso M, Asnaghi V, Restagno G, Mandrile G, Botta G, Silengo MC, Grosso E, Ferrero

GB, Brusco A. Large cryptic genomic rearrangements with apparently normal karyotypes detected by array-CGH. *Mol Cytogenet*. 2014;7(1):82. doi:10.1186/s13039-014-0082-7

232. Shaffer LG, Dabell MP, Fisher AJ, Coppinger J, Bandholz AM, Ellison JW, Ravnan JB, Torchia BS, Ballif BC, Rosenfeld JA. Experience with microarray-based comparative genomic hybridization for prenatal diagnosis in over 5000 pregnancies: Microarray experience in over 5000 pregnancies. *Prenat Diagn*. 2012;32(10):976-985. doi:10.1002/pd.3945

233. Muys J, Blaumeiser B, Janssens K, Loobuyck P, Jacquemyn Y. Chromosomal microarray analysis in prenatal diagnosis: ethical considerations of the Belgian approach. *J Med Ethics*. 2020;46(2):104-109. doi:10.1136/medethics-2018-105186

234. Zou Z, Huang L, Lin S, He Z, Zhu H, Zhang Y, Fang Q, Luo Y. Prenatal diagnosis of posterior fossa anomalies: Additional value of chromosomal microarray analysis in fetuses with cerebellar hypoplasia. *Prenat Diagn*. 2018;38(2):91-98. doi:10.1002/pd.5190

235. Guterman S, Beneteau C, Redon S, Dupont C, Missirian C, Jaeger P, Herve B, Jacquin C, Douet-Guilbert N, Till M, Tabet A, Moradkhani K, Malan V, Doco-Fenzy M, Vialard F. Prenatal findings in 1p36 deletion syndrome: New cases and a literature review. *Prenat Diagn*. 2019;39(10):871-882. doi:10.1002/pd.5498

236. de Wit MC, Srebniak MI, Govaerts LCP, Van Opstal D, Galjaard RJH, Go ATJI. Additional value of prenatal genomic array testing in fetuses with isolated structural ultrasound abnormalities and a normal karyotype: a systematic review of the literature:

Genomic microarray testing in fetuses with structural anomalies. *Ultrasound Obstet Gynecol*. 2014;43(2):139-146. doi:10.1002/uog.12575

237. Gardiner C, Wellesley D, Kilby MD, Kerr B, on bejalf of the Joint Committee on Genomics in Medicine. *Recommendations for the Use of Chromosome Microarray in Pregnancy.*; 2015.

238. Schmid M, Stary S, Blaicher W, Gollinger M, Husslein P, Streubel B. Prenatal genetic diagnosis using microarray analysis in fetuses with congenital heart defects: Congenital heart defects and microarray analysis. *Prenat Diagn*. 2012;32(4):376-382. doi:10.1002/pd.2862

239. Chen M, Yang YS, Shih JC, Lin WH, Lee DJ, Lin YS, Chou CH, Cameron AD, Ginsberg NA, Chen CA, Lee ML, Ma GC. Microdeletions/duplications involving *TBX1* gene in fetuses with conotruncal heart defects which are negative for 22q11.2 deletion on fluorescence *in-situ* hybridization: TBX1 microdeletions/duplications in fetal CTD. *Ultrasound Obstet Gynecol*. 2014;43(4):396-403. doi:10.1002/uog.12550

240. Erdogan F, Larsen LA, Zhang L, Tümer Z, Tommerup N, Chen W, Jacobsen JR, Schubert M, Jurkatis J, Tzschach A, Ropers HH, Ullmann R. High frequency of submicroscopic genomic aberrations detected by tiling path array comparative genome hybridisation in patients with isolated congenital heart disease. *J Med Genet*. 2008;45(11):704-709. doi:10.1136/jmg.2008.058776

241. Syrmou A, Tzetis M, Fryssira H, Kosma K, Oikonomakis V, Giannikou K, Makrythanasis P, Kitsiou-Tzeli S, Kanavakis E. Array comparative genomic hybridization

as a clinical diagnostic tool in syndromic and nonsyndromic congenital heart disease. *Pediatr Res*. 2013;73(6):772-776. doi:10.1038/pr.2013.41

242. Wu X li, Li R, Fu F, Pan M, Han J, Yang X, Zhang Y ling, Li F tao, Liao C. Chromosome microarray analysis in the investigation of children with congenital heart disease. *BMC Pediatr*. 2017;17(1):117. doi:10.1186/s12887-017-0863-3

243. Breckpot J, Thienpont B, Peeters H, de Ravel T, Singer A, Rayyan M, Allegaert K, Vanhole C, Eyskens B, Vermeesch JR, Gewillig M, Devriendt K. Array Comparative Genomic Hybridization as a Diagnostic Tool for Syndromic Heart Defects. *J Pediatr*. 2010;156(5):810-817.e4. doi:10.1016/j.jpeds.2009.11.049

244. Lin M, Zheng J, Peng R, Du L, Zheng Q, Lei T, Xie H. Prenatal diagnosis of chromosomal aberrations in fetuses with conotruncal heart defects by genome-wide high-resolution SNP array. *J Matern Fetal Neonatal Med*. 2020;33(7):1211-1217. doi:10.1080/14767058.2018.1517316

245. Barron DJ, Kilby MD, Davies B, Wright JG, Jones TJ, Brawn WJ. Hypoplastic left heart syndrome. *The Lancet*. 2009;374(9689):551-564. doi:10.1016/S0140-6736(09)60563-8

246. Hitz MP, Lemieux-Perreault LP, Marshall C, Feroz-Zada Y, Davies R, Yang SW, Lionel AC, D'Amours G, Lemyre E, Cullum R, Bigras JL, Thibeault M, Chetaille P, Montpetit A, Khairy P, Overduin B, Klaassen S, Hoodless P, Nemer M, Stewart AFR, Boerkoel C, Scherer SW, Richter A, Dubé MP, Andelfinger G. Rare Copy Number Variants Contribute to Congenital Left-Sided Heart Disease. Spinner NB, ed. *PLoS Genet*. 2012;8(9):e1002903. doi:10.1371/journal.pgen.1002903

247. Gómez O, Martínez JM, Olivella A, Bennasar M, Crispi F, Masoller N, Bartrons J, Puerto B, Gratacós E. Isolated ventricular septal defects in the era of advanced fetal echocardiography: risk of chromosomal anomalies and spontaneous closure rate from diagnosis to age of 1 year: Outcome of isolated fetal VSD. *Ultrasound Obstet Gynecol*. 2014;43(1):65-71. doi:10.1002/uog.12527

248. Du L, Xie H ning, Li L juan, Zhu Y xiao, Lin M fang, Zheng J. [Association between fetal ventricular septal defects and chromosomal abnormalities]. *Zhonghua Fu Chan Ke Za Zhi*. 2013;48(11):805-809.

249. Dolk H, Loane M, Garne E, a European Surveillance of Congenital Anomalies (EUROCAT) Working Group. Congenital Heart Defects in Europe: Prevalence and Perinatal Mortality, 2000 to 2005. *Circulation*. 2011;123(8):841-849. doi:10.1161/CIRCULATIONAHA.110.958405

250. Papp C, Beke A, Mezei G, Szigeti Z, Bán Z, Papp Z. Prenatal Diagnosis of Turner Syndrome: Report on 69 Cases. *J Ultrasound Med*. 2006;25(6):711-717. doi:10.7863/jum.2006.25.6.711

251. Veugelers M. Mutational analysis of the GPC3/GPC4 glypican gene cluster on Xq26 in patients with Simpson-Golabi-Behmel syndrome: identification of loss-of-function mutations in the GPC3 gene. *Hum Mol Genet*. 2000;9(9):1321-1328. doi:10.1093/hmg/9.9.1321

252. Yano S, Baskin B, Bagheri A, Watanabe Y, Moseley K, Nishimura A, Matsumoto N, Ray P. Familial Simpson-Golabi-Behmel syndrome: studies of X-chromosome

inactivation and clinical phenotypes in two female individuals with GPC3 mutations. *Clin Genet*. 2011;80(5):466-471. doi:10.1111/j.1399-0004.2010.01554.x

253. Brantberg A, Blaas HGK, Haugen SE, Eik-Nes SH. Characteristics and outcome of 90 cases of fetal omphalocele: Fetal omphalocele. *Ultrasound Obstet Gynecol*. 2005;26(5):527-537. doi:10.1002/uog.1978

254. Shi X, Tang H, Lu J, Yang X, Ding H, Wu J. Prenatal genetic diagnosis of omphalocele by karyotyping, chromosomal microarray analysis and exome sequencing. *Ann Med*. 2021;53(1):1286-1292. doi:10.1080/07853890.2021.1962966

255. Lloveras E, Pérez C, Solé F, Zamora L, Lladonosa A, Espinet B, Silvestre E, Serra J, Vendrell T, Fernández B, Salido M, Plaja A. Two cases of tetrasomy 9p syndrome with tissue limited mosaicism: Partial Tetrasomy 9p: Two New Cases. *Am J Med Genet A*. 2004;124A(4):402-406. doi:10.1002/ajmg.a.20447

256. Chen CP, Wang LK, Chern SR, Wu PS, Chen YT, Kuo YL, Chen WL, Lee MS, Wang W. Mosaic tetrasomy 9p at amniocentesis: Prenatal diagnosis, molecular cytogenetic characterization, and literature review. *Taiwan J Obstet Gynecol*. 2014;53(1):79-85. doi:10.1016/j.tjog.2013.12.002

257. Fleurke-Rozema JH, van de Kamp K, Bakker MK, Pajkrt E, Bilardo CM, Snijders RJM. Prevalence, diagnosis and outcome of cleft lip with or without cleft palate in The Netherlands. *Ultrasound Obstet Gynecol*. 2016;48(4):458-463. doi:10.1002/uog.15834

258. de wit MC, Srebniak MI, Govaerts LCP, Van Opstal D, Galjaard RJH, Go ATJI. Additional value of prenatal genomic array testing in fetuses with isolated structural ultrasound abnormalities and a normal karyotype: a systematic review of the literature:

Genomic microarray testing in fetuses with structural anomalies. *Ultrasound Obstet Gynecol*. 2014;43(2):139-146. doi:10.1002/uog.12575

259. Maarse W, Rozendaal AM, Pajkrt E, Vermeij-Keers C, Mink van der Molen AB, van den Boogaard MJH. A systematic review of associated structural and chromosomal defects in oral clefts: when is prenatal genetic analysis indicated? *J Med Genet*. 2012;49(8):490-498. doi:10.1136/jmedgenet-2012-101013

260. Yoshida J, Tsuchiya M, Tatsuma N, Murakami M. Mass screening for early detection of congenital kidney and urinary tract abnormalities in infancy. *Pediatr Int*. 2003;45(2):142-149. doi:10.1046/j.1442-200X.2003.01681.x

261. Nakanishi K, Yoshikawa N. Genetic disorders of human congenital anomalies of the kidney and urinary tract (CAKUT). *Pediatr Int*. 2003;45(5):610-616. doi:10.1046/j.1442-200X.2003.01779.x

262. Sanna-Cherchi S, Kiryluk K, Burgess KE, Bodria M, Sampson MG, Hadley D, Nees SN, Verbitsky M, Perry BJ, Sterken R, Lozanovski VJ, Materna-Kiryluk A, Barlassina C, Kini A, Corbani V, Carrea A, Somenzi D, Murtas C, Ristoska-Bojkovska N, Izzi C, Bianco B, Zaniew M, Flogelova H, Weng PL, Kacak N, Giberti S, Gigante M, Arapovic A, Drnasin K, Caridi G, Curioni S, Allegri F, Ammenti A, Ferretti S, Goj V, Bernardo L, Jobanputra V, Chung WK, Lifton RP, Sanders S, State M, Clark LN, Saraga M, Padmanabhan S, Dominiczak AF, Foroud T, Gesualdo L, Gucev Z, Allegri L, Latos-Bielenska A, Cusi D, Scolari F, Tasic V, Hakonarson H, Ghiggeri GM, Gharavi AG. Copy-Number Disorders Are a Common Cause of Congenital Kidney Malformations. *Am J Hum Genet*. 2012;91(6):987-997. doi:10.1016/j.ajhg.2012.10.007

263. Westland R, Verbitsky M, Vukojevic K, Perry BJ, Fasel DA, Zwijnenburg PJG, Bökenkamp A, Gille JJP, Saraga-Babic M, Ghiggeri GM, D'Agati VD, Schreuder MF, Gharavi AG, van Wijk JAE, Sanna-Cherchi S. Copy number variation analysis identifies novel CAKUT candidate genes in children with a solitary functioning kidney. *Kidney Int*. 2015;88(6):1402-1410. doi:10.1038/ki.2015.239

264. Verbitsky M, Sanna-Cherchi S, Fasel DA, Levy B, Kiryluk K, Wuttke M, Abraham AG, Kaskel F, Köttgen A, Warady BA, Furth SL, Wong CS, Gharavi AG. Genomic imbalances in pediatric patients with chronic kidney disease. *J Clin Invest*. 2015;125(5):2171-2178. doi:10.1172/JCI80877

265. Kobrynski LJ, Sullivan KE. Velocardiofacial syndrome, DiGeorge syndrome: the chromosome 22q11.2 deletion syndromes. *The Lancet*. 2007;370(9596):1443-1452. doi:10.1016/S0140-6736(07)61601-8

266. Portnoï MF. Microduplication 22q11.2: A new chromosomal syndrome. *Eur J Med Genet*. 2009;52(2-3):88-93. doi:10.1016/j.ejmg.2009.02.008

267. Rey RA, Codner E, Iñíguez G, Bedecarrás P, Trigo R, Okuma C, Gottlieb S, Bergadá I, Campo SM, Cassorla FG. Low Risk of Impaired Testicular Sertoli and Leydig Cell Functions in Boys with Isolated Hypospadias. *J Clin Endocrinol Metab*. 2005;90(11):6035-6040. doi:10.1210/jc.2005-1306

268. Geller F, Feenstra B, Carstensen L, Pers TH, van Rooij IALM, Körberg IB, Choudhry S, Karjalainen JM, Schnack TH, Hollegaard MV, Feitz WFJ, Roeleveld N, Hougaard DM, Hirschhorn JN, Franke L, Baskin LS, Nordenskjöld A, van der Zanden LFM, Melbye M.

Genome-wide association analyses identify variants in developmental genes associated with hypospadias. *Nat Genet*. 2014;46(9):957-963. doi:10.1038/ng.3063

269. Epelboym Y, Estrada C, Estroff J. Ultrasound diagnosis of fetal hypospadias: Accuracy and outcomes. *J Pediatr Urol*. 2017;13(5):484.e1-484.e4. doi:10.1016/j.jpurol.2017.02.022

270. Nemec SF, Nemec U, Brugger PC, Bettelheim D, Weber M, Graham JM, Rimoin DL, Prayer D. Male genital abnormalities in intrauterine growth restriction: Male genital abnormalities in intrauterine growth restriction. *Prenat Diagn*. 2012;32(5):427-431. doi:10.1002/pd.3831

271. Fuchs F, Borrego P, Amouroux C, Antoine B, Ollivier M, Faure JM, Lopez C, Forgues D, Faure A, Merrot T, Boulot P, Jeandel C, Philibert P, Gaspari L, Sultan C, Paris F, Kalfa N. Prenatal imaging of genital defects: clinical spectrum and predictive factors for severe forms. *BJU Int*. 2019;124(5):876-882. doi:10.1111/bju.14714

272. Longoni M, Lage K, Russell MK, Loscertales M, Abdul-Rahman OA, Baynam G, Bleyl SB, Brady PD, Breckpot J, Chen CP, Devriendt K, Gillessen-Kaesbach G, Grix AW, Rope AF, Shimokawa O, Strauss B, Wieczorek D, Zackai EH, Coletti CM, Maalouf FI, Noonan KM, Park JH, Tracy AA, Lee C, Donahoe PK, Pober BR. Congenital diaphragmatic hernia interval on chromosome 8p23.1 characterized by genetics and protein interaction networks. *Am J Med Genet A*. 2012;158A(12):3148-3158. doi:10.1002/ajmg.a.35665

273. Shi P, Wang C, Zheng Y, Kong X. Prenatal and postnatal diagnoses and phenotype of 8p23.3p22 duplication in one family. *BMC Med Genomics*. 2021;14(1):88. doi:10.1186/s12920-021-00940-z

274. Barber JCK, Maloney VK, Huang S, Bunyan DJ, Cresswell L, Kinning E, Benson A, Cheetham T, Wyllie J, Lynch SA, Zwolinski S, Prescott L, Crow Y, Morgan R, Hobson E. 8p23.1 duplication syndrome; a novel genomic condition with unexpected complexity revealed by array CGH. *Eur J Hum Genet*. 2008;16(1):18-27. doi:10.1038/sj.ejhg.5201932

275. Kalsner L, Chamberlain SJ. Prader-Willi, Angelman, and 15q11-q13 Duplication Syndromes. *Pediatr Clin North Am*. 2015;62(3):587-606. doi:10.1016/j.pcl.2015.03.004

276. Cox D, Butler M. The 15q11.2 BP1–BP2 Microdeletion Syndrome: A Review. *Int J Mol Sci*. 2015;16(2):4068-4082. doi:10.3390/ijms16024068

277. Pajkrt E, Chitty LS. A sonographic approach to the prenatal diagnosis of skeletal dysplasias. *Prenat Diagn*. 2019;39(9):701-719. doi:10.1002/pd.5501

278. Barkova E, Mohan U, Chitayat D, Keating S, Toi A, Frank J, Frank R, Tomlinson G, Glanc P. Fetal skeletal dysplasias in a tertiary care center: radiology, pathology, and molecular analysis of 112 cases: Fetal skeletal dysplasias in a tertiary care center. *Clin Genet*. 2015;87(4):330-337. doi:10.1111/cge.12434

279. Royer-Bertrand B, Castillo-Taucher S, Moreno-Salinas R, Cho TJ, Chae JH, Choi M, Kim OH, Dikoglu E, Campos-Xavier B, Girardi E, Superti-Furga G, Bonafé L, Rivolta C, Unger S, Superti-Furga A. Mutations in the heat-shock protein A9 (HSPA9) gene cause the EVEN-PLUS syndrome of congenital malformations and skeletal dysplasia. *Sci Rep*. 2015;5(1):17154. doi:10.1038/srep17154

280. Dikoglu E, Alfaiz A, Gorna M, Bertola D, Chae JH, Cho TJ, Derbent M, Alanay Y, Guran T, Kim OH, Llerenar Jr JC, Yamamoto G, Superti-Furga G, Reymond A, Xenarios I, Stevenson B, Campos-Xavier B, Bonafé L, Superti-Furga A, Unger S. Mutations in *LONP1* , a mitochondrial matrix protease, cause CODAS syndrome. *Am J Med Genet A*. 2015;167(7):1501-1509. doi:10.1002/ajmg.a.37029

281. Malone F. Isolated clubfoot diagnosed prenatally: is karyotyping indicated? *Obstet Gynecol*. 2000;95(3):437-440. doi:10.1016/S0029-7844(99)00582-7

282. Wang H, Barisic I, Loane M, Addor MC, Bailey LM, Gatt M, Klungsoyr K, Mokoroa O, Nelen V, Neville AJ, O'Mahony M, Pierini A, Rissmann A, Verellen-Dumoulin C, de Walle HEK, Wiesel A, Wisniewska K, de Jong-van den Berg LTW, Dolk H, Khoshnood B, Garne E. Congenital clubfoot in Europe: A population-based study. *Am J Med Genet A*. 2019;179(4):595-601. doi:10.1002/ajmg.a.61067

283. Xing Y, Holder JL, Liu Y, Yuan M, Sun Q, Qu X, Deng L, Zhou J, Yang Y, Guo M, Cheung SW, Sun L. Prenatal diagnosis of Wolf–Hirschhorn syndrome: from ultrasound findings, diagnostic technology to genetic counseling. *Arch Gynecol Obstet*. 2018;298(2):289-295. doi:10.1007/s00404-018-4798-1

284. Battaglia A, Carey JC, Wright TJ. Wolf-Hirschhorn (4p-) syndrome. *Adv Pediatr*. 2001;48:75-113.

285. Ikonomou T, Antsaklis P, Daskalakis G, Sindos M, Papantoniou N, Kosmaidou Z, Antsaklis A. Prenatal diagnosis of Wolf–Hirschhorn syndrome: ultrasonography and genetics. *J Matern Fetal Neonatal Med*. 2013;26(9):941-942. doi:10.3109/14767058.2013.765855

286. Heinonen S, Ryynänen M, Kirkinen P. Etiology and outcome of second trimester non-immunologic fetal hydrops. *Acta Obstet Gynecol Scand*. 2000;79(1):15-18.

287. Santo S, Mansour S, Thilaganathan B, Homfray T, Papageorghiou A, Calvert S, Bhide A. Prenatal diagnosis of non-immune hydrops fetalis: what do we tell the parents?: PERINATAL OUTCOME OF NON-IMMUNE HYDROPS. *Prenat Diagn*. 2011;31(2):186-195. doi:10.1002/pd.2677

288. Parchem JG, Sparks TN, Gosnell K, Norton ME. Utility of chromosomal microarray in anomalous fetuses. *Prenat Diagn*. 2018;38(2):140-147. doi:10.1002/pd.5202

289. Mardy AH, Chetty SP, Norton ME, Sparks TN. A system-based approach to the genetic etiologies of non-immune hydrops fetalis. *Prenat Diagn*. 2019;39(9):732-750. doi:10.1002/pd.5479

290. Deng Q, Fu F, Yu Q, Li R, Li F, Wang D, Lei T, Yang X, Liao C. Nonimmune hydrops fetalis: Genetic analysis and clinical outcome. *Prenat Diagn*. 2020;40(7):803-812. doi:10.1002/pd.5691

PART 10

PUBLICATIONS

10 PUBLICATIONS

Paper number 1:

Santirocco M, Plaja A, Rodó C, Valenzuela I, Arévalo S, Castells N, Abuli A, Tizzano E, Maiz N, Carreras E. Chromosomal microarray analysis in fetuses with central nervous system anomalies: An 8-year long observational study from a tertiary care university hospital. Prenat Diagn. 2021 Jan;41(1):123-135. doi: 10.1002/pd.5829. Epub 2020 Sep 30. PMID: 32926442.

Received: 30 June 2020 | Revised: 1 September 2020 | Accepted: 8 September 2020

DOI: 10.1002/pd.5829

ORIGINAL ARTICLE

PRENATAL DIAGNOSIS WILEY

Chromosomal microarray analysis in fetuses with central nervous system anomalies: An 8-year long observational study from a tertiary care university hospital

Maddalena Santirocco[1,2] | Alberto Plaja[2,3] | Carlota Rodó[1,2] | Irene Valenzuela[3] | Silvia Arévalo[1,2] | Neus Castells[2,3] | Anna Abuli[2,3] | Eduardo Tizzano[2,3] | Nerea Maiz[1,2] | Elena Carreras[1,2]

[1]Maternal-Fetal Medicine Department, Obstetrics Department, Vall d'Hebron Hospital Universitari, Barcelona, Spain

[2]Universitat Autònoma de Barcelona, Bellaterra, Spain

[3]Department of Clinical and Molecular Genetics and Medicine Genetics Group, VHIR, Vall d'Hebron Hospital Universitari, Barcelona, Spain

Correspondence
Nerea Maiz, Maternal-Fetal Medicine Department, Obstetrics Department, Hospital Universitari Vall d'Hebron, Passeig Vall d'Hebron 119-129, 08035 Barcelona, Spain.
Email: nmaiz@vhebron.net

Abstract

OBJECTIVES: To evaluate the prevalence of DNA copy number variants (CNVs) detected with array comparative genomic hybridization (CGH) in fetuses with central nervous system (CNS) anomalies. Secondary objectives were to describe the prevalence of CNV in specific CNS abnormalities, in isolated defects or associated with other malformations or fetal growth restriction (FGR).

METHODS: Observational cohort study in 238 fetuses with CNS anomalies in which an array-CGH had been performed between January 2009 and December 2017. Pathogenic CNV and variants of unknown significance (VUS) were reported.

RESULTS: Pathogenic CNVs were found in 16/238 cases (6.7%), VUS in 18/238 (7.6%), and normal result in 204/238 (85.7%) cases. Pathogenic CNVs were more frequent in posterior fossa anomalies (cerebellar hypoplasia 33%, megacisterna magna 20%), moderate ventriculomegaly (11%) and spina bifida (3.7%). Pathogenic CNVs and VUS were found in 7/182 (3.8%) and 14/182 (7.7%) cases of isolated anomalies, in 9/49 (18.4%) and 4/49 (8.2%) presenting another malformation, and in 0/7 and 0/7 cases with associated FGR (P = .001, P = .741, respectively).

CONCLUSION: These results provide strong evidence toward performing array in fetuses with CNS anomalies, particular in cases of posterior fossa anomalies. The prevalence of pathogenic CNVs is higher in association with other malformations.

1 | INTRODUCTION

Central Nervous System (CNS) anomalies cover a broad spectrum of disorders that can be present either in an isolated form or associated with other extra-cerebral alterations. They comprise brain anomalies and/or neural tube defects.[1]

The exact incidence of CNS anomalies in European countries is uncertain, it is reported to vary from 1.3 to 3 per 1000 live births in prenatal studies,[2] however long term follow up studies suggest an incidence as high as 1 in 100 live births.[1] Studies on stillbirths report a prevalence up to 3% to 6%.[2]

The etiology of CNS alterations is very heterogeneous and genetic conditions are recognized as an important causing factor. Known disease-causing genetic factors include chromosomal abnormalities (eg, trisomy 18, Trisomy 13, Miller Dieker syndrome) and monogenic syndromes (eg, holoprosencephaly type 3, Joubert syndrome).[3] However, the underlying cause of most cerebral anomalies is still unknown.

Karyotyping has been considered the gold standard method for the detection of chromosomal abnormalities. However, its resolution is limited to around 5 to 10 Mb depending on (a) the location of the genome analyzed, (b) the quality of the chromosome preparation and (c) the skill and experience of the cytogeneticist.[4]

Chromosomal microarray analysis (CMA) allows studying the whole genome searching for DNA copy number variants (CNVs), as small as 50 to 100 Kb, well below the resolution of a standard karyotype.[4,5] Array-comparative genomic hybridization (CGH) is one of the most widely used CMA techniques. Array-CGH compares DNA content from two differentially labeled genomes with a resolution limited only by the size of the DNA probes immobilized in the array and the natural distance between these sequences located on the chromosome.

Several studies have investigated the use of array analysis in prenatal settings, in fetuses presenting abnormal ultrasound findings.[6,7] CMA provides additional information over karyotype in about 6% to 7% of pregnancies presenting an anomaly identified by ultrasound, most frequently cardiac, renal, skeletal, urogenital, and CNS anomalies.[8,9]

Nevertheless, although pathogenic CNVs play a significant role in the etiology of CNS abnormalities,[3,10,11] this association has been barely described in the literature.

The objectives of this study were to evaluate the prevalence of CNVs of pathogenic and uncertain significance (VUS) in fetuses with CNS anomalies, to describe the association with specific CNS anomalies and to describe the association with both other structural abnormalities and fetal growth restriction.

What's already known about this topic?

- Pathogenic copy number variants (CNVs) play a significant role in the etiology of central nervous system anomalies, although this association has been barely described in the literature.

What does this study add?

- In a series of 238 fetuses with central nervous system anomalies, pathogenic CNV were found in 6.7% of the cases.
- The specific isolated anomalies with the highest prevalence of pathogenic CNVs were posterior fossa abnormalities, moderate ventriculomegaly, and spina bifida.

2 | METHODS

This is an observational study performed at the Department of Maternal-Fetal Medicine in a collaborative effort with the Department of Clinical and Molecular Genetics of the Vall d'Hebron University Hospital in Barcelona, Spain, between January 2009 and December 2017. Ethical approval for this study was provided by the *Comité de ética de investigación con medicamentos* (CEIm) from the Vall d'Hebron University Hospital, Barcelona, Spain. The study population was composed of pregnant women with fetuses presenting CNS anomalies detected during prenatal ultrasound in which an array-CGH had also been performed. Exclusion criteria were abnormal quantitative fluorescence-polymerase chain reaction (QF-PCR) for chromosomes 21, 18, 13 or sex chromosomes and fetal infections (Cytomegalovirus, toxoplasma, Zika).

The following data was collected: maternal age, gestational age at the moment of performance of the invasive test, type of invasive test (chorionic villus sampling, amniocentesis, fetal blood or fetal tissue biopsy), QF-PCR, array-CGH results, associated structural anomalies and the presence of fetal growth restriction (FGR).

2.1 | Clinical protocol

Women were referred to the Fetal Medicine Unit of our hospital following the finding of a CNS anomaly. A detailed fetal ultrasound and a neurosonography were performed following ISUOG guidelines.[11] A magnetic resonance imaging (MRI) was requested in selected cases. An invasive test (either amniocentesis or chorionic villous sampling, as appropriate by gestational age) was offered in all cases. In those women that declined invasive testing and opted for termination of pregnancy, a post-mortem array-CGH study from a fetal sample was offered to the parents. In all cases of invasive testing a pre- and post-test genetic counselling was offered.

From the beginning of the implementation of microarray, the policy of our hospital has always been to report only pathogenic or probably pathogenic CNVs in the prenatal setting. Women were informed that unless they stated otherwise they would not be informed of CNVs of benign or uncertain significance. All CNVs have been reviewed again and uncertain CNVs have been taken into account for the purpose of the study only.

2.2 | Genetic testing

Firstly, a QF-PCR for chromosomes 21, 18, 13, X, and Y was carried out. The array-CGH study was then performed in all cases in which the QF-PCR was normal.

Array-CGH technique: DNA was extracted from uncultured or cultured samples of amniotic fluid and chorion biopsies using the iGENatal genomic DNA extraction Kit (igenBiotech, Madrid) and subsequently analyzed with QF-PCR Devyser Complete kit (Devyser, Sweden), following the recommendations of the manufacturers. If QF-PCR detected any aneuploidy, karyotype analysis was performed to confirm the result and discard structural alterations, otherwise fetal DNA was analyzed with CytoSure Constitutional 8×60 K v3 (ogt, UK) or qChip Pre 8×60 K (qgenomics, Spain) array CGH assays following the recommendations of the manufacturers. Both arrays have mixed designs, with a backbone of an average resolution of 350 to 663 Kb and a higher resolution (of 100-375 Kb) in regions associated to pathology. Ogt arrays has exonic resolution in 354 genes selected by the ClinGen Dosage Sensitivity Map.[12] In case 1, because low quality of DNA sample, a custom made low resolution BAC array was utilized.

Array-CGH results evaluation: CNVs were classified following recommendations of the American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants (in brief, rare recessive variants not related to fetal phenotypic abnormalities and CNVs classified as benign were not reported).[13,14] Additionally, to reduce the anxiety of pregnant couples (an informed consent was obtained in all the cases), findings of uncertain significance, and of low penetrance were not reported. VUS is defined as a CNV described in multiple contradictory publications and/or databases, and firm conclusions regarding clinical significance are not yet established. While this study was ongoing, some international scientific societies published similar recommendations.[13,15-18] All variants, reported or not, were included in our analysis with the only exception of benign CNVs or VUS of smaller than 400 kb.

Except for some well-known recurrent structural abnormalities known to be always "de novo," parents of all fetuses with pathogenic or probably pathogenic CNVs were proposed to be investigated with karyotype, BAC FISH or array-CGH to evaluate a possible recurrence risk. With VUS or variables of low penetrance, not reported by our reporting policy, parental samples were investigated only if available or findings were communicated after the termination of pregnancy.

The results were given in a specific consultation of post-test genetic counselling explaining the results and the implications crucial to support informed decision-making.

2.3 | Statistical analysis

For the descriptive analysis categorical variables were described as an absolute number and percentage, while continuous data as a median and interquartile (IQR) range.

The chi-square test was used to compare the prevalence of pathogenic CNV and VUS among isolated cases, cases with associated abnormalities and associated FGR.

3 | RESULTS

Two hundred and forty-four cases with CNS anomalies and array-CGH study were identified. From these, six cases were excluded, three had an abnormal QF-PCR (one case each of trisomy 21, 18 and 13) and three were diagnosed with a fetal infection (one case of cytomegalovirus and two cases of Zika virus). Two hundred and thirty-eight cases were therefore included in the analysis (Figure 1).

3.1 | Demographic characteristics

Median maternal age was 33 years (IQR, 29-36), median gestational age at invasive testing was 21.5 weeks (IQR, 20-25). One hundred and twenty-eight women (53.8%) were nulliparous, and 110 (46.2%) were multiparous. Two hundred and twenty-two (93.3%) were singleton pregnancies, 12 (5%) were dichorionic twin pregnancies, and 4 (1.7%) were monochorionic twin pregnancies.

3.2 | Type of anomaly

Anomalies detected included ventriculomegaly (n = 83, 34.9%); neural tube defects (n = 62, 26.1%); midline anomalies (n = 42, 17.6%); posterior fossa anomalies (n = 31, 13.0%); cortical development anomalies (n = 13, 5.5%); hypoxic-ischemic or hemorrhagic lesions (n = 2, 0.8%),

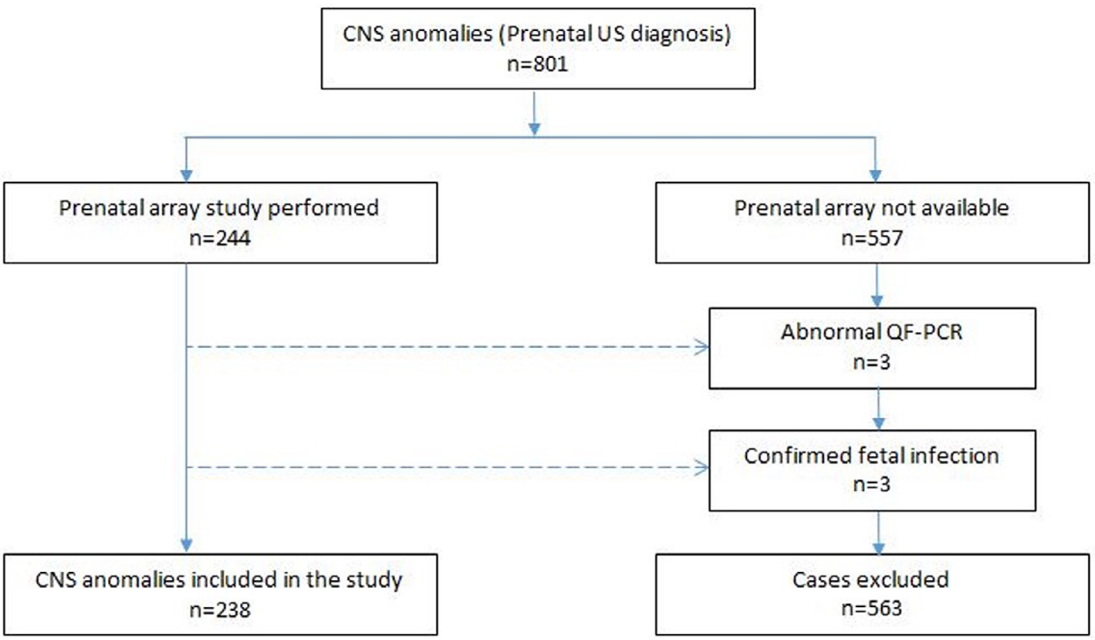


FIGURE 1 Flowchart: selection criteria [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Prevalence of pathogenic copy number variants (CNVs) and VUS in the different types of CNS anomalies

	ALL (n = 238)			ISOLATED (n = 182)			COMPLEX ABNORMALITIES (n = 49)		
	N	Pathogenic CNVs	VUS	N	Pathogenic CNVs	VUS	N	Pathogenic CNVs	VUS
Ventriculomegaly	83/238 (34.9%)	7/83 (8.4%)	6/83 (7.2%)	63/182 (34.7%)	3/63 (4.8%)	4/63 (6.3%)	16/49 (32.7%)	4/16 (25.0%)	2/16 (12.5%)
Borderline/Mild	57/83 (68.7%)	3/57 (5.3%)	3/57 (5.3%)	46/63 (73%)	2/46 (4.3%)	2 /46 (4.3%)	8/16 (50%)	1/8 (12.5%)	1/8 (12.5%)
Moderate	12/83 (14.5%)	3/12 (25%)	1/12 (8.3%)	9/63 (14.3%)	1/9 (11.1%)	1 /9 (11.1%)	2/16 (12.5%)	2/2 (100%)	-
Severe	14/83 (16.9%)	1/14 (7.1%)	2/14 (14.3%)	8/63 (12.7%)	0 (0%)	1 (12.5%)	6/16 (37.5%)	1/6 (16.7%)	1/6 (16.7%)
Neural tube defects	62/238 (26.1%)	2/62 (3.2%)	3/62 (4.8%)	58 (31.89%)	2/58 (3.4%)	2/58 (3.4%)	4/49 (8.2%)	0/4 (0%)	1/4 (25%)
Acrania	2/62 (3.2%)	0 (0%)	1 (50%)	1/58 (1.7%)	0 (0%)	0 (0%)	1/4 (25%)	0	1/4 (25%)
Encephalocele	3/62 (3.2%)	0 (0%)	1 (33.3%)	3/58 (5.2%)	0 (0%)	1 (33.3%)	-	-	-
Spina bifida	57/62 (93.5%)	2/57 (3.5%)	1/57 (1.8%)	54/58 (93%)	2/54 (3.7%)	1/54 (1.9%)	3/4 (75%)	0 (0%)	0 (0%)
Midline anomalies	42/238 (17.6%)	2/42 (4.8%)	3/42 (7.1%)	32 (17.6%)	0/32 (0%)	3/32 (9.4%)	10/49 (20.4%)	2/10 (20%)	0/10 (0%)
Complete agenesis corpus callosum	22/42 (52.4%)	0 /22(0%)	1 /22 (4.5%)	18/32 (56.3%)	0 (0%)	1/18 (5.6%)	4/10 (40%)	0 (0%)	0 (0%)
Partial agenesis / dysgenesis corpus callosum	10/42 (23.8%)	0/10 (0%)	1/10 (10%)	8/32 (25%)	0/8 (0%)	1/8 (12.5%)	2/10 (20%)	0 (0%)	0(0%)
Holoprosencephaly	6/42 (14.3%)	2/6 (33.3%)	1/6 (16.7%)	3/32 (9.4%)	0 (0%)	1/3 (33.3%)	3/10 (30%)	2/3 (66.7%)	0
CSP agenesis	4/42 (9.5%)	0 (0%)	0 (0%)	3/32 (9.4%)	0 (0%)	0 (0%)	1/10 (10%)	0	0
Posterior fossa anomalies	31/238 (13.0%)	4/31 (12.9%)	4/31 (12.9%)	17 (9.3%)	2/17 (11.8%)	3/17 (17.6%)	13/49 (26.5%)	2/13 (15.4%)	1/13 (7.7%)
Dandy Walker malformation	5/31 (16.1%)	1/5 (20%)	1/5 (20%)	3/17 (17.6%)	0 (0%)	1 (33.3%)	2/13 (15.4%)	1/2 (50%)	0
Cerebellar hypoplasia	6/31 (19.4%)	1/6 (16.7%)	0 (0%)	3/17 (17.6%)	1/3 (33.3%)	0 (0%)	3/13 (23.1%)	0	0
Vermian hypoplasia	2/31 (6.5%)	0 (0%)	0 (0%)	0 (0%)	-	-	2/13 (15.4%)	0	0
Megacisterna magna	8/31 (25.8%)	2/8 (25%)	1/8 (12.5%)	5/17 (29.4%)	1/5 (20%)	0 (0%)	2/13 (15.4%)	1/2 (50%)	1
Blake's pouch cyst	6/31 (19.4%)	0 (0%)	0/6 (0%)	3/17 (17.6%)	0 (0%)	0/3 (0%)	3/13 (23.1%)	0	0
Posterior fossa cyst	1/31 (3.2%)	0 (0%)	0 (0%)	1/17 (5.9%)	0 (0%)	0 (0%)	-	-	-
Rhombencephalosynapsis	3/31 (9.7%)	0 (0%)	2/3 (66.7%)	2/17 (11.8%)	0 (0%)	2/2 (100%)	1/13 (7.7%)	0	0
Cortical development anomalies	13/238 (5.5%)	1/13 (7.7%)	2/13 (15.4%)	6/182 (3.3%)	0 (0%)	2/6 (33.3%)	5/49 (10.2%)	1/5 (20%)	0/5 (0%)
Macrocephaly	2/13 (15.4%)	0 (0%)	0 (0%)	1/6 (16.7%)	0 (0%)	0 (0%)	1/5 (20%)	0	0
Polymycrogyria	2/13 (21.4%)	0 (0%)	1 /2 (50%)	1/6 (16.7%)	0 (0%)	1/1 (100%)	1/5 (20%)	0	0
Abnormal sulcation/gyration	4/13 (30.8%)	1/4 (25%)	1/4 (25%)	2/6 (33%)	0 (0%)	1/2 (50%)	1/5 (20%)	1/1 (100%)	0
Microcephaly	5/13 (38.5%)	0 (0%)	0 (0%)	2/6 (33.3%)	0 (0%)	0 (0%)	2/5 (40%)	0	0
Hypoxic-ischemic or haemorrhagic lesion	2/238 (0.8%)	0/2 (0%)	0/2 (0%)	2 (1.1%)	0 (0%)	0 (0%)	-	-	-
Brain haemorrhage	1 /2 (50%)	0 (0%)	0 (0%)	1/ 2 (50%)	0 (0%)	0 (0%)	-	-	-
Venous sinus thrombosis	1 /2 (50%)	0 (0%)	0 (0%)	1/ 2 (50%)	0 (0%)	0 (0%)	-	-	-
Intracranial cyst	3/238 (1.3%)	0 (0%)	0 (0%)	3 (1.6%)	0 (0%)	0 (0%)	-	-	-

TABLE 1 (Continued)

	ALL (n = 238)			ISOLATED (n = 182)			COMPLEX ABNORMALITIES (n = 49)		
	N	Pathogenic CNVs	VUS	N	Pathogenic CNVs	VUS	N	Pathogenic CNVs	VUS
Arachnoid cyst	3/3 (100%)	0 (0%)	0 (0%)	3/3 (100%)	0 (0%)	0 (0%)	-	-	-
Periventricular hyperechogenicity	1 (0.4%)	0 (0%)	0 (0%)	1 (0.5%)	0 (0%)	0 (0%)	-	-	-
Tumor	1 (0.4%)	0 (0%)	0 (0%)	0 (0%)	-	-	1/49 (2%)	0/1 (0%)	0/1 (0%)

intracranial cysts (n = 3, 1.3%); brain tumor (n = 1, 0.4%) and periventricular hyperechogenicity (n = 1, 0.4%). Additional major non-CNS anomalies were detected in 49 cases (20.6%), including congenital heart defects (n = 25, 10.5%); facial dysmorphisms (n = 10, 4.2%); thoracic anomalies (n = 8, 3.4%); gastrointestinal or abdominal wall anomalies (n = 7, 2.9%); renal anomalies (n = 7, 2.9%); skeletal anomalies (n = 10, 4.2%); fetal hydrops (n = 2, 0.8%) and abnormal genitalia (n = 8, 3.4%). Minor ultrasound anomalies were found in 31 cases (13%).

Fetal growth restriction was diagnosed in 16 cases (6.7%), of which nine had also major abnormalities. In 182 cases (76.5%), an isolated CNS anomaly was detected.

3.3 | Array-CGH study

In 225 (94.5%) cases an amniocentesis was performed, in 10 (4.2%) a chorionic villous sampling and in three cases (1.3%) fetal tissue for chromosomal analysis was obtained following termination of pregnancy.

A pathogenic CNV was diagnosed in 16 cases (6.7%), VUS in 18 cases (7.6%), including two cases of probably pathogenic (CNVs that meet some but not all criteria to be considered pathogenic), and a normal result in 204 (85.7%) cases. Table 1 show pathogenic CNV and VUS according to the type and the subgroup of CNS anomaly detected, either isolated or associated with other anomalies, respectively. A pathogenic CNV was found in 7 of the 182 (3.8%) cases of isolated anomalies, in 9 of the 49 (18.4%) that presented another major anomaly, and in none of the seven cases with associated FGR (P = .001). A VUS was found in 14 of the 182 (7.7%) with an isolated anomaly, in 4 (8.2%) of the 49 with associated major anomalies and in none of the seven cases with FGR (P = .741).

Considering the isolated cases with pathogenic CNVs or VUS that opted for a termination of pregnancy (in the case of VUS because ultrasound findings), of the 14 prenatally isolated cases, in the post-mortem examinations were found: one case of additional cerebral anomaly (case number 33 presented an inferior vemis hypoplasia), and one case of extracerebral anomaly (case number 29 presented a double vagina). For those cases that opted for TOP with a normal array in which a post-mortem exam is available: of the 53 prenatally isolated cases without CNVs, in seven cases additional cerebral o extracerebral anomalies were found in the post-mortem examination (see Tables S1 and S2 for details).

The individual description of each case with pathogenic CNV and VUS is provided in Tables 2 and 3.

4 | DISCUSSION

4.1 | Main findings

We investigated the prevalence of pathogenic CNVs and VUS detected by CGH-Array in a cohort of 238 fetuses with different CNS

TABLE 2 Details of all cases with pathogenic CNVs

Case number	Comments	Gestational age (weeks)	CNS anomaly	Other anomalies	array	Chromosome region and/or genetic content associated with the phenotype	De novo/ inherited	Test	Potentially visible by karyotype analysis	Follow up
1	BAC based array with low resolution	22	Ventriculomegaly	Pericardial effusion and right heart hypertrophy	6q26q27(163240985_ 170861575)x1 (7.54 Mb) 8p23.2p23.1(145466_ 7169549)x3 (7.02 Mb) 8p23.1(11269493_ 11998276)x3 (723 Kb)	Chromosome recurrent anomaly inv dup del(8p): ventriculomegaly.[31] CNS function and development genes DLGAP2 (OMIM 605438), CLN8 (OMIM 607837), ARHGEF10 (OMIM 608136). Congenital heart defects genes GATA4 (OMIM 600576) [31]	De novo	qChip Pre v1.1 Targeted	YES	TOP
2		16	Ventriculomegaly	Congenital diaphragmatic hernia, renal cyst	Xq26.2(132315039 _132876911) x0 (0.56 Mb)	Ventriculomegaly, congenital diaphragmatic hernia, renal cyst: Simpson-Golabi-Behmel Syndrome Type 1. Genes, GPC4 (OMIM: 300168), GPC3 (OMIM: 300037)	Maternal	Agilent G4827A (CGH ISCA v2,8x60K)	NO	TOP
3		18	Dandy Walker malformation	Hypoplastic left heart with double outlet right ventricle; Cystic higroma	1q21.1q21.2 (145415190-147380935) x3 1.96 Mb)	Complete 1q21.1 duplication (proximal + distal). q21 distal (BP3-BP4) duplication show variable penetrance of 17-47% and its known to be associated to brain and heary anomalies[32]	Paternal	Agilent G4827A (CGH ISCA v2,8x60K)	NO	TOP
4		20	Cerebellar hypoplasia	-	6q27(169099035_ 170911240)x1 (1.91 Mb)	Region associated to brain malformations[33] especially periventricular nodular heterotopia (PNH) [34]	Paternal balanced translocation (46,XY,t(6;13) (q27;p11.2)	qChip Pre v1.1 Complete	NO	TOP
5		21	Holoprosencephaly	Oligosyndactlyly; Fetal growth restriction	13q31.3q34(91571035_ 115093115)x1 (23.5 Mb)	ZIC2 (OMIM 603073) 8.2% of holoprosencephaly (HPE) probands show ZIC2 (MIM603073) defects, mostly "de novo" (70% of cases) [35]. The overall penetrance of phenotypic manifestations (including microform HPE) due to mutations in ZIC2 is estimated to be 96% and the prevalence of brain anomalies is estimated to be 90% [36]. ZIC2 mutations are generally characterized by a normal face or a moderate facial dysmorphia associated with alobar or semilobar HPE [35].	De novo	ogt 020045 (CytoSure Constitutional v3 array8x60K)	YES	TOP

TABLE 2 (Continued)

Case number	Comments	Gestational age (weeks)	CNS anomaly	Other anomalies	array	Chromosome region and/or genetic content associated with the phenotype	De novo/ inherited	Test	Potentially visible by karyotype analysis	Follow up
6		20	Abnormal sulcation	Cleft lip	1p36.33p36.32(794596_4458182)x1 (3.57 Mb)	Brain anomalies and orofacial clefting are part of the 1p36 deletion syndrome [37].	De novo	qChip Pre v1.1 Targeted	NO	TOP
7		20	Megacisterna magna	Nuchal edema	3q29(193892289_196215670)x3 (2.3 Mb)	Enlarged cisterna magna has been described in 3q29 syndrome.[38]	Maternal	qChip Pre	NO	Severe developmental delay and epilepsy (4 years)
8		25	Ventriculomegaly	-	5q21.3q23.1 (105029588_120102372)x1 (15 Mb)	5q deletion includng APC (OMIM:611731) gene is associated to risk of adenomatous polyposis, (MIM175100) and a variable and a phenotypically poor defined phenotipic syndrome including dysmorphic features, and mild mental retardation [39].	De novo	qChip Pre v1.1 Complete	YES	TOP
9		17	Megacisterna magna	Pulmonary stenosis, hydrothorax, ascites; hyperechogenic kidneys; micrognathia	9p24.3p13.1 (204090_38704041)x1 (38.5 Mb)	DMRT1 DOCK8 Small telomeric 9p24.3 deletions including DMRT genes (DMRT1 bring the strongest candidate gene for sex reversal) cause genital anomalies in male subjects, ranging from disorder of gonadal sex to genital differentiation anomalies. More proximal, interstitial 9p22.3-p24.1 deletions result in a malformation syndrome characterized by intellectual disability, congenital hypotonia and a range of cranio-facial abnormalities, less frequently cardiac defects, epilepsy, inguinal hernia, omphalocele, choanal atresia, scoliosis and non-ketotic hypoglycaemia [40].	De novo	- qChip Pre v1.1 Complete	YES	TOP
10		25	Spina bifida	-	15q25.2q25.3 (84084071_86870834)x1 (2.8 Mb)	15q25.2 distal deletions should be considered as a susceptibility locus for variable neurodevelopmental disorders with high risk than proximal deletions for neuropsychiatric disorders, seizures, hypotonia, and strabism. One patient has dysmorphic features similar to Noonan syndrome[41].	De novo	qChip Pre v1.1 Targeted	NO	Normal neurodevelopment

(Continues)

TABLE 2 (Continued)

Case number	Comments	Gestational age (weeks)	CNS anomaly	Other anomalies	array	Chromosome region and/or genetic content associated with the phenotype	De novo/ inherited	Test	Potentially visible by karyotype analysis	Follow up
11		27	Ventriculomegaly	-	10q11.22q11.23 (47596461_ 51798957)x1 (4.4 Mb)	10q11.2 deletions are associated to brain abnormlities: 19% patients described have microcephaly and 18%, corpus callosum abnormalities [42]	unknown origin	qChip Pre v1.1 Complete	NO	No follow up
12		32	Ventriculomegaly	Hidrops; Polihydramnios	17q12(34856055_ 36777884)x1 (1.9 Mb)	The 17q12 recurrent deletion syndrome is characterized by structural or functional abnormalities of the kidney and urinary tract (80-85% affected individuals) [43]	Declined study	qChip Pre v1.1 Complete	NO	Neonatal death
13		24	Ventriculomegaly	-	5q35.2 _ q35.3(175667946_ 177422740)x3 (1.75 Mb)		De novo	ogt 020045 (CytoSure Constitutional v3 array (8x60K)	NO	Live birth, no follow up
14		25	Holoprosencephaly	Ventricular septum defect; Fetal growth restriction	13q31.3 _ q34(92587094_ 115093115)x1 (22.5 Mb)	Deletion includes ZIC2 (MIM603073). Defects in ZIC2 cause holoprosencephaly (HPE) [35].	De novo	ogt 020045 (CytoSure Constitutional v3 array 8x60K)	YES	TOP
15	Not mosaicism evidence in chorion villus array and karyotype analysis.	13	Hydrocephaly; abnormal posterior fossa	Cardiac defect (not specified); Exomphalos; Nuchal edema	(9)x3	Mosaic trisomy 9 is a rare Nuchal abnormalities, central nervous system, and cardiac are common findings in trisomy of chromosome 9 [44]	Not studied	ogt 020045 (CytoSure Constitutional v3 array8x60K)	YES	TOP
16		18	Neural tube defect (spina bifida)	-	Xq27.1(139131377_ 139724080)x2 (592Kb)	SOX3 (OMIM: 313430) duplication has been recently associated with neural tube defects.[26,27]	Not studied	qChip Pre Complete	NO	TOP

TABLE 3 Details of all cases with VUS

Case number	Type of VUS†	Gestational age (weeks)	CNS anomaly	Other anomalies	Array	Affected genes	De novo/ inherited	Test	Potentially visible by karyotype analysis	Follow up
17		13.3	Alobar Holoprosencephaly	-	4p16.3(45889_127278)x3(81 Kb)	ZNF595, ZNF718	Not studied	ogt 020045 (CytoSure Constitutional v3 array 8x60K	NO	TOP
18		14	encephalocele	Cystic higroma	10q11.22(46699438_49263598) x3 (2.56 Mb)	26 genes	Not studied	Agilent G4827A (CGH ISCA v2,8x60K	NO	TOP
19		20	Hidrocephaly		1q21.2(150619114_151174820)x3 (556 Kb) Arr 7p22.2(2759448_ 2803743)x1 (44 Kb)	CTKS,ARNT,MLLT1 GNA12	Paternal (both CNVs)	qChip post	NO	TOP
20	†	21	Ventriculomegaly	Tetralogy of fallot; microretrognathia; clubfoot	2q21.2(134067405_ 134295740)x1 (228 Kb) 14q11.2(22409129_ 23167893)x3 (759 Kb)	NAP5 DAD1,ABHD4	Not studied	qChip post		TOP
21		30	Ventriculomegaly	-	2p16.1(55281597_ 56633505)x3 (1.35 Mb)	13 genes	Maternal	Agilent G4827A (CGH ISCA v2, 8x60K	NO	Normal neurodevelopment 1 year FO
22	†	21	Abnormal sulcation	Ventriculomegaly	3q29(196263081_196825905)x3 (0.56 Mb) (VUS because includes only 50% of 3q29 duplication syndrome)	12 genes (PAK2)	Paternal (normal phenotype)	020045 (CytoSure Constitutional v3 array 8x60K	NO	TOP
23		30	Ventriculomegaly	Fetal growth restriction,	Xp22.31(7744144_8435991)x2 (540 Kb)	NA	Not studied	qChip pre	NO	Ventriculomegaly; brainstem disgenesis; complex cardiopathy
24		Post TOP	Agenesis corpus callosum	Polymicrogyria	7q21.3(93039264_ 93234125)x1 (195 Kb)	CALCR	Not studied	qChip post	NO	TOP
25		29	Ventriculomegaly	-	Xp22.13(18609619_ 19030114)x2 (420 Kb)	RS1, PPEF1, PHKA2, RefSeq (CDKL5,GPR64)	Maternal	qChip Pre Complete	NO	Partial syndactyly foot; Normal neurodevelopment FO 7 months
26		18	Dandy Walker (vermian agenesis)	-	Xq21.31(87877030_ 88253336)x2 (376 Kb)	CPXCR1	Maternal	qChip post	NO	TOP
27		28	Megacisterna magna	Tricuspid valve dysplasia	11q14.1(81827270_ 83453945)x3 (1.6 Mb) 17p11.2(19572563_ 19790651)x3 (218 Kb)	DLG2, PCRP, RAB30,ANKRD42 ALDH3A2, SLC47A2, ALDH3A1, ULK2	Maternal	qChip Pre v1.1 Targeted	NO	Neuronal heterotopias
28		20	Anencephaly	Ventricular septum defect; single umbilical artery	16p13.11(15551302_ 16194578)x3 (643 Kb)	C16orf45, KIAA0430, NDE1, MIR484, MYH11, C16orf63, ABCC1	Not studied	Agilent G4827A (CGH ISCA v2, 8x60K	NO	TOP

(Continues)

TABLE 3 (Continued)

Case number	Type of VUS†	Gestational age (weeks)	CNS anomaly	Other anomalies	Array	Affected genes	De novo/ inherited	Test	Potentially visible by karyotype analysis	Follow up
29		21	Neural tube defect (myelocele)	-	Xp22.31(7810940_ 8434361)x2 (623 Kb)	VCX, PNPLA4, MIR651, VCX2, VCX3B	Not studied	ogt 020045 (CytoSure Constitutional v3 array8x60K	NO	TOP
30		25	rhombencephalosynapsis	-	Xq21.1(77082384_77139044)x3 (57 Kb)	MAGT1	Not studied	ogt 020045 (CytoSure Constitutional v3 array 8x60K	NO	Rhombencephalosynapsis (suspect Gomez-Lopez-Hernandez Snd)
31		21	rhombencephalosynapsis	-	11q24.3(128170803_ 128554700)x3 (384 Kb)	ETS1	Not studied	ogt 020045 (CytoSure Constitutional v3 array 8x60K	NO	TOP
32		27	Abnormal sulcation	-	17p13.3(810138_912611) x1 (102 Kb)	NXN, TIMM22, ABR	Not studied	ogt 020045 (CytoSure Constitutional v3 array 8x60K	NO	TOP
33		22	Ventriculomegaly	-	12q14.3(65564724_65671221) x3 (106 Kb)	LEMD3	Not studied	ogt 020045 (CytoSure Constitutional v3 array 8x60K	NO	TOP
34		22	Partial agenesis of corpus callosum	-	13q12.11q12.12(20100790_ 25458788)x1 (5.3 Mb)	>30	De novo	qChip Pre v1.1 Targeted	doubtful	TOP

anomalies. Our results showed a similar prevalence of 7% for CNVs and 8% of VUS once aneuploidies of chromosomes 13, 18, 21, X, and Y were excluded. The prevalence of pathogenic CNVs was higher when other structural abnormalities were present (18.4% of cases with an associated anomaly vs 3.8% of cases of isolated anomalies). On the other hand, VUS showed similar results (8.2% if associated anomalies were present vs 7.7% if the detected anomaly was isolated), reflecting the probable lack of relevant phenotypic effect of most of the VUS and, and adding evidence in favor of the policy of not reporting them prenatally.[15,16] Interestingly, when isolated anomalies involved either the posterior fossa or cortical development, a higher prevalence of pathogenic CNVs and VUS was observed. In isolated CNS anomalies the malformations with a higher prevalence of pathogenic CNVs were cerebellar hypoplasia (33%), megacisterna magna (20%), moderate ventriculomegaly (11%) and spina bifida (3.7%).

4.2 | Results in the context of what is known

The use of CMA in the prenatal setting has proven to be an excellent tool compared to standard cytogenetic karyotyping in the diagnosis of chromosomal anomalies in case of fetuses carrying one or more major malformations and a normal karyotype.[7,13] Several studies have reported the prevalence of pathogenic CNVs and VUS in fetuses with ultrasound anomalies. The prevalence of pathogenic CNVs in these cases is about 5% to 7%.[6,9,13,17] However, fewer studies have explicitly reported on CNS anomalies,[23-26] the prevalence of pathogenic CNVs in these studies in fetuses with a normal karyotype varies between 3.7% and 10.9%. Our results show a similar prevalence of pathogenic CNVs, 4.2% (10 out of 238) if only CNVs below the resolution of karyotype analysis are considered.

We found six cases of pathogenic CNVs that would have been potentially visibles also if a standard karyotype had been performed (abnormalities larger than 5-10 Mb).[27] (Details are reported in Tables 2 and 3).

The highest prevalence of pathogenic CNVs in our study was found for posterior fossa anomalies (12.9%) being cerebellar hypoplasia the most prevalent (33%), followed by ventriculomegaly (8.4%). These data are consistent with the available literature.[10,21] Zou et al report a prevalence of 10.8% in cases of posterior fossa anomalies, with the highest prevalence in those cases of cerebellar hypoplasia (25%), vermian hypoplasia, and Dandy Walker anomal.[22]

There are broad differences in the prevalence of VUS described in the literature for several reasons. First, and besides the type of array employed (CGH, SNPs BACs), different reporting policies exist in different countries and laboratories. Therefore, VUS might be under-reported, since most studies may have considered only patient-informed VUS, but not all detected VUS. Second, as in pathogenic CNVs, there might be a selection bias in those studies performed on patients who underwent termination of pregnancy. Third, reported cohorts involving CNS anomalies usually involve smaller cohorts than our study, being more prone to random fluctuations. Our study has

taken into account all detected CNVs, whether reported or not, in a cohort of 238 fetuses presenting CNS anomalies, and we found a higher prevalence than other studies. A prevalence of 4.8% has been reported in a more extensive general study involving 2858 pregnancies with ultrasound anomalies[17] and of 5.2% in a metanalysis involving 799 fetuses with ultrasound anomalies.[13] Lower diagnostic yield of both studies can be easily explained by the inclusion of a significant proportion of BAC based array-CGH analysis, an low resolution technology currently replaced by the oligonucleotide based technology used in our study. Considering literature data focusing on CNS anomalies, our results are comparable to those of Sun et al reporting a prevalence of VUS in CNS anomalies of 6.5% in 46 subjects,[23] but lower than the prevalence reported by Schumann et al with a 27% of VUS in 33 fetal samples derived from termination of pregnancy.[24] However, the high frequency in the later study could reflect fluctuations due to the relatively low number of fetuses included.

Some recommendations about the use of microarray in the prenatal setting suggest not to inform about alterations that are not clearly associated to pathogenic phenotypes in current literature.[15,16] Not all the groups working in the prenatal field agree with this clinical conduct since this could be considered a paternalistic vision in an era where the self-determination of patients is arising; this is especially true in the case of a fetus whose legal definition changes across different countries. On the other way to report prenatally all the variants could generate anxiety in the parents and physicians in those cases in which it has to be decided about the future of an unborn child, in a moment in which is not possible to confirm exactly the phenotype.[21] Regarding the specific involvement of genes, SOX3 duplication has been recently associated with neural tube defects.[28,29] In our series we found SOX3 duplication in a male fetus affected with spina bifida.

We also detected a de novo 1p36 deletion in a fetus showing abnormal cerebral sulcation associated to cleft lip. A recent series of cases with 1p36 deletion suggests association to brain and facial abnormalities and reinforcing the indication of CMA study when these prenatal findings are detected. The authors postulate that 1p36 deletion is difficult to diagnose given that it can be present without specific ultrasound signs.[30]

4.3 | Limitations of the study

Our study is retrospective and based only on pregnancies that underwent an invasive test. It is likely that some women declined an invasive test in cases of mild or CNS anomalies, and we do not have the postnatal follow up of those children.

4.4 | Clinical implications

Our study provides further evidence to recommend the use of MCA studies that in our opinion should be the first-tier test over karyotype, multiple ligation-dependent probe amplification and fluorescence in situ hybridization techniques for prenatal diagnosis.[13,19]

The large sample size analyzed allowed us to perform a subanalysis for each type of anomaly, providing more detailed information regarding the prevalence of pathogenic CNVs and VUS in specific brain anomalies detected prenatally.

In our opinion, this study could help to better define the prognosis of CNS anomalies especially those considered isolated in prenatal diagnosis and could help to reclassify in the future some VUS through the association between fetal anomalies and the arising evidence about genetic alterations.

We stress the importance of a collaborative basis between the Obstetrics and Genetics Departments for direct access to the complete CMA data analysis allowing to find in our study a higher prevalence of VUS, frequently omitted in final reports of CMA results in prenatal setting.[20]

5 | CONCLUSION

The results of our study provide strong evidence toward performing array tests in case of CNS anomalies. This is especially true in those cases of posterior fossa abnormalities. The prevalence of pathogenic CNVs in those cases with associated anomalies is higher than in isolated CNS anomalies.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Nerea Maiz https://orcid.org/0000-0001-8261-6383

REFERENCES

1. Chitty LS, Pilu G. The challenge of imaging the fetal central nervous system: an aid to prenatal diagnosis, management and prognosis. *Prenat Diagn*. 2009;29(4):301-302.
2. Onkar D. Evaluation of fetal central nervous system anomalies by ultrasound and its anatomical co-relation. *J Clin Diagn Res*. 2014;8(6): AC05-AC07.
3. Jeng LB, Tarvin R, Robin NH. Genetic advances in central nervous system malformations in the fetus and neonate. *Semin Pediatr Neurol*. 2001;8(2):89-99.
4. Wapner RJ, Martin CL, Levy B, et al. Chromosomal microarray versus karyotyping for prenatal diagnosis. *N Engl J Med*. 2012;367(23):2175-2184.
5. Bui T-H, Vetro A, Zuffardi O, Shaffer LG. Current controversies in prenatal diagnosis 3: is conventional chromosome analysis necessary in the post-array CGH era? *Prenat Diagn*. 2011;31(3):235-243.
6. Lovrecic L, Remec ZI, Volk M, Rudolf G, Writzl K, Peterlin B. Clinical utility of array comparative genomic hybridisation in prenatal setting. *BMC Med Genet*. 2016;17(1):81.
7. D'Amours G, Kibar Z, Mathonnet G, et al. Whole-genome array CGH identifies pathogenic copy number variations in fetuses with major malformations and a normal karyotype. *Clin Genet*. 2012;81(2): 128-141.

8. Levy B, Wapner R. Prenatal diagnosis by chromosomal microarray analysis. *Fertil Steril*. 2018;109(2):201-212.
9. Srebniak MI, Boter M, Oudesluijs GO, et al. Genomic SNP array as a gold standard for prenatal diagnosis of foetal ultrasound abnormalities. *Mol Cytogenet*. 2012;5(1):14.
10. Shaffer LG, Dabell MP, Fisher AJ, et al. Experience with microarray-based comparative genomic hybridization for prenatal diagnosis in over 5000 pregnancies: microarray experience in over 5000 pregnancies. *Prenat Diagn*. 2012;32(10):976-985.
11. International Society of Ultrasound in Obstetrics & Gynecology Education Committee. Sonographic examination of the fetal central nervous system: guidelines for performing the 'basic examination' and the 'fetal neurosonogram'. *Ultrasound Obstet Gynecol*. 2007;29(1): 109-116.
12. ClinGen Genome Dosage Map. https://dosage.clinicalgenome.org/. Accessed August 30, 2020.
13. Hillman SC, Pretlove S, Coomarasamy A, et al. Additional information from array comparative genomic hybridization technology over conventional karyotyping in prenatal diagnosis: a systematic review and meta-analysis. *Ultrasound Obstet Gynecol*. 2011;37(1):6-14.
14. Riggs ER, Andersen EF, Cherry AM, et al. Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the clinical genome resource (ClinGen). *Genet Med*. 2020;22(2):245-257.
15. Muys J, Blaumeiser B, Jacquemyn Y, et al. The Belgian MicroArray prenatal (BEMAPRE) database: a systematic nationwide repository of fetal genomic aberrations. *Prenat Diagn*. 2018;38(13):1120-1128.
16. Vanakker O, Vilain C, Janssens K, et al. Implementation of genomic arrays in prenatal diagnosis: the Belgian approach to meet the challenges. *Eur J Med Genet*. 2014;57(4):151-156.
17. Shaffer LG, Rosenfeld JA, Dabell MP, et al. Detection rates of clinically significant genomic alterations by microarray analysis for specific anomalies detected by ultrasound: microarray experience with abnormal ultrasound anomalies. *Prenat Diagn*. 2012;32(10):986-995.
18. The Royal of Pathologists, BSGM, Royal College of Obstetricians & Gynaecologists. Recommendations for the use of chromosome microarray in pregnancy. Published June 2015. https://www.rcpath.org/uploads/assets/06664c28-0f90-4230-86158c91fea14be6/Recommendations-for-the-use-of-chromosome-microarray-in-pregnancy.pdf. Accessed August 30, 2020.
19. de Wit MC, Srebniak MI, Govaerts LCP, et al. Additional value of prenatal genomic array testing in fetuses with isolated structural ultrasound abnormalities and a normal karyotype: a systematic review of the literature: genomic microarray testing in fetuses with structural anomalies. *Ultrasound Obstet Gynecol*. 2014;43(2):139-146.
20. Gardiner C, Wellesley D, Kilby MD, Kerr B, on bejalf of the Joint Committee on Genomics in Medicine. Recommendations for the Use of Chromosome Microarray in Pregnancy. 2015.
21. Muys J, Blaumeiser B, Janssens K, Loobuyck P, Jacquemyn Y. Chromosomal microarray analysis in prenatal diagnosis: ethical considerations of the Belgian approach. *J Med Ethics*. 2020;46(2):104-109.
22. Zou Z, Huang L, Lin S, et al. Prenatal diagnosis of posterior fossa anomalies: additional value of chromosomal microarray analysis in fetuses with cerebellar hypoplasia. *Prenat Diagn*. 2018;38(2): 91-98.
23. Sun L, Wu Q, Jiang S-W, et al. Prenatal diagnosis of central nervous system anomalies by high-resolution chromosomal microarray analysis. *Biomed Res Int*. 2015;2015:426379.
24. Schumann M, Hofmann A, Krutzke SK, et al. Array-based molecular karyotyping in fetuses with isolated brain malformations identifies disease-causing CNVs. *J Neurodev Disord*. 2016;8(1):11.
25. Krutzke SK, Engels H, Hofmann A, et al. Array-based molecular karyotyping in fetal brain malformations: identification of novel candidate genes and chromosomal regions: CNV analyses of nonisolated CNS malformations. *Birt Defects Res A Clin Mol Teratol*. 2016;106(1): 16-26.
26. Li Z, Fu F, Lei T, et al. Application of chromosome microarray analysis for the delineation of pathogenesis for fetal ventriculomegaly. Zhonghua Yi Xue Yi Chuan Xue Za Zhi Zhonghua Yixue Yichuanxue Zazhi Chin. *J Med Genet*. 2017;34(4):576-582.
27. Di Gregorio E, Savin E, Biamino E, et al. Large cryptic genomic rearrangements with apparently normal karyotypes detected by array-CGH. *Mol Cytogenet*. 2014;7(1):82.
28. Uguen A, Talagas M, Quémener-Redon S, Marcorelles P, de Braekeleer M. Duplication of SOX3 (Xq27) may be a risk factor for neural tube defects. *Am J Med Genet A*. 2015;167(7):1676-1678.
29. Hureaux M, Ben Miled S, Chatron N, et al. SOX3 duplication: a genetic cause to investigate in fetuses with neural tube defects. *Prenat Diagn*. 2019;39(11):1026-1034.
30. Guterman S, Beneteau C, Redon S, et al. Prenatal findings in 1p36 deletion syndrome: new cases and a literature review. *Prenat Diagn*. 2019; 39(10):871-882.
31. Peddibhotla S, Nagamani SCS, Erez A, et al. Delineation of candidate genes responsible for structural brain abnormalities in patients with terminal deletions of chromosome 6q27. *Eur J Hum Genet*. 2015;23 (1):54–60. http://dx.doi.org/10.1038/ejhg.2014.51.
32. Chen CP, Ko TM, Huang WC, et al. Molecular cytogenetic characterization of inv dup del(8p) in a fetus associated with ventriculomegaly, hypoplastic left heart, polyhydramnios and intestinal obstruction. *Taiwan J Obstet Gyne*. 2016;55(3):415–418. http://dx.doi.org/10.1016/j.tjog.2016.05.001.
33. Onesimo R, Orteschi D, Scalzone M, et al. Chromosome 9p deletion syndrome and sex reversal: Novel findings and redefinition of the critically deleted regions. *Am J Med Genet Part A*. 2012;158A(9):2266–2271. http://dx.doi.org/10.1002/ajmg.a.35489.
34. O'Driscoll MC, Black GCM, Clayton-Smith J, Sherr EH, Dobyns WB. Identification of genomic loci contributing to agenesis of the corpus callosum. *Am J Med Genet Part A*. 2010;152A(9):2145–2159. http://dx.doi.org/10.1002/ajmg.a.33558.
35. Garcia-Miñaur S, Ramsay J, Grace E, Minns RA, Myles LM, FitzPatrick DR. Interstitial deletion of the long arm of chromosome 5 in a boy with multiple congenital anomalies and mental retardation: Molecular characterization of the deleted region to 5q22.3q23.3. *Am J Med Genet Part A*. 2005;132A(4):402–410. http://dx.doi.org/10.1002/ajmg.a.30421.
36. Mercier S, Dubourg C, Garcelon N, et al. New findings for phenotype-genotype correlations in a large European series of holoprosencephaly cases. *J Med Genet*. 2011;48(11):752–760. http://dx.doi.org/10.1136/jmedgenet-2011-100339.
37. Kor-anantakul O, Suwanrath C, Kanngurn S, Rujirabanjerd S, Suntharasaj T, Pinjaroen S. Prenatal Diagnosis of Complete Trisomy 9: A Case Report and Review of the Literature. *Am J Perinatol*. 2006; 23 (02):131–136. http://dx.doi.org/10.1055/s-2006-931804.
38. Doelken SC, Seeger K, Hundsdoerfer P, Weber-Ferro W, Klopocki E, Graul-Neumann L. Proximal and distal 15q25.2 microdeletions-genotype-phenotype delineation of two neurodevelopmental susceptibility loci. *Am J Med Genet Part A*. 2013;161(1):218–224. http://dx.doi.org/10.1002/ajmg.a.35695.
39. Stankiewicz P, Kulkarni S, Dharmadhikari AV, et al. Recurrent deletions and reciprocal duplications of 10q11.21q11.23 including CHAT and SLC18A3 are likely mediated by complex low-copy repeats. *Hum Mutat*. 2012;33(1):165–179. http://dx.doi.org/10.1002/humu.21614.
40. Scott D, Jordan V, Zaveri H. 1p36 deletion syndrome: an update. *Appl Clin Genet*. 2015;189. http://dx.doi.org/10.2147/tacg.s65698.
41. Solomon BD, Lacbawan F, Mercier S, et al. Mutations in ZIC2 in human holoprosencephaly: description of a Novel ZIC2 specific phe-

notype and comprehensive analysis of 157 individuals. *J Med Genet*. 2010;47(8):513–524. http://dx.doi.org/10.1136/jmg.2009.073049.
42. Goobie S, Knijnenburg J, FitzPatrick D, et al. Molecular and clinical characterization of de novo and familial cases with microduplication 3q29: guidelines for copy number variation case reporting. *Cytogenet Genome Res*. 2008;123(1-4):65–78. http://dx.doi.org/10.1159/000184693.
43. 17q12 deletion syndrome. *RAREDISEASES.INFO.NIH.GOV*. https://rarediseases.info.nih.gov/diseases/13297/disease.
44. Verhagen Judith MA, de Leeuw N, Papatsonis Dimitri NM, Grijseels Els WM, de Krijger Ronald R., Wessels Marja W. Phenotypic Variability Associated with a Large Recurrent 1q21.1 Microduplication in a Three-Generation Family. *Mol Syndromol*. 2015;6(2):71–76. http://dx.doi.org/10.1159/000431274.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: Santirocco M, Plaja A, Rodó C, et al. Chromosomal microarray analysis in fetuses with central nervous system anomalies: An 8-year long observational study from a tertiary care university hospital. *Prenatal Diagnosis*. 2020;1–13. https://doi.org/10.1002/pd.5829

Paper number 2:

Santirocco M, Rodó C, Illescas T, Vázquez É, Garrido M, Higueras T, Arévalo S, Maiz N, Carreras E. Accuracy of prenatal ultrasound in the diagnosis of corpus callosum anomalies. J Matern Fetal Neonatal Med. 2021 Feb;34(3):439-444. doi: 10.1080/14767058.2019.1609931. Epub 2019 Apr 29. PMID: 31035852..

The Journal of Maternal-Fetal & Neonatal Medicine

ISSN: 1476-7058 (Print) 1476-4954 (Online) Journal homepage: https://www.tandfonline.com/loi/ijmf20

Accuracy of prenatal ultrasound in the diagnosis of corpus callosum anomalies

Maddalena Santirocco, Carlota Rodó, Tamara Illescas, Élida Vázquez, Marta Garrido, Teresa Higueras, Silvia Arévalo, Nerea Maiz & Elena Carreras

To cite this article: Maddalena Santirocco, Carlota Rodó, Tamara Illescas, Élida Vázquez, Marta Garrido, Teresa Higueras, Silvia Arévalo, Nerea Maiz & Elena Carreras (2019): Accuracy of prenatal ultrasound in the diagnosis of corpus callosum anomalies, The Journal of Maternal-Fetal & Neonatal Medicine, DOI: 10.1080/14767058.2019.1609931

To link to this article: https://doi.org/10.1080/14767058.2019.1609931

THE JOURNAL OF MATERNAL-FETAL & NEONATAL MEDICINE
https://doi.org/10.1080/14767058.2019.1609931

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

Accuracy of prenatal ultrasound in the diagnosis of corpus callosum anomalies

Maddalena Santirocco[a], Carlota Rodó[a], Tamara Illescas[a,b], Élida Vázquez[c], Marta Garrido[d], Teresa Higueras[a], Silvia Arévalo[a], Nerea Maiz[a] and Elena Carreras[a]

[a]Department of Maternal–Fetal Medicine, Hospital Universitari Vall d'Hebron, Universitat Autónoma de Barcelona, Barcelona, Spain; [b]Department of Obstetrics and Gynecology, Hospital Universitario La Paz, Madrid, Spain; [c]Department of Paediatric Radiology, Hospital Universitari Vall d'Hebron, Barcelona, Spain; [d]Department of Pathology, Hospital Universitari Vall d'Hebron, Barcelona, Spain

ABSTRACT

Objectives: The main objective of this study was to evaluate the accuracy of prenatal ultrasound to diagnose corpus callosum alterations, compared to prenatal magnetic resonance imaging (MRI), postnatal image techniques (ultrasound and/or MRI), and post-mortem examination in terminated pregnancies.

Methods: Retrospective review of 86 cases of prenatal ultrasound diagnosis of corpus callosum anomalies between January 2007 and December 2015 at a third level Maternal Fetal Medicine center. The study reviewed the findings of prenatal ultrasound and MRI, post-mortem examination in cases of termination of pregnancy (TOP) or stillbirths and postnatal ultrasound, and MRI in neonates. The anomalies of corpus callosum (CC) were classified as complete agenesis of the corpus callosum (ACC), partial ACC, or dysgenesis of CC.

Results: Fifty-eight (67.4%) cases resulted in TOP, 26 (30.2%) cases opted to continue with the pregnancy and two (2.3%) cases were lost to follow up. Among the 26 cases that continued with the pregnancy, 24 (92.3%) were live births and two (7.7%) were stillborn. All cases in which a third trimester MRI was performed ($n = 46$) confirmed the prenatal ultrasound diagnosis of CC anomaly. In seven (15.2%) of them, the MRI found additional intracranial findings and in three cases (6.5%) the type of CC anomaly (complete, partial, or dysgenesis) was reclassified (Kappa index: 0.86, 95% CI: 0.71–1.00). CC anomalies were confirmed in 46 (95.8%) of the 48 cases in which a post-mortem examination was available, the type of anomaly being reclassified in three cases (6.3%) (Kappa index: 0.88, 95% CI: 0.75–1.00). Among the 10 cases in which a postnatal ultrasound was performed, the CC anomaly was confirmed in all and the type of anomaly was reclassified in 1 (10%) of them (Kappa index: 0.75, 95% CI: 0.32–1.00).

Conclusion: Corpus callosum agenesis can be detected on the routine mid-trimester ultrasound scan. Prenatal ultrasound and MRI can accurately classify the type of CC abnormality. Moreover, third trimester MRI can detect additional intracranial anomalies in 15% of cases.

ARTICLE HISTORY
Received 5 February 2019
Revised 4 April 2019
Accepted 17 April 2019

KEYWORDS
Corpus callosum agenesis; corpus callosum dysgenesis; magnetic resonance imaging; post-mortem examination; prenatal; ultrasound

Introduction

The corpus callosum (CC) is the main telencephalic commissure of the human brain for interhemispheric communication [1]. It permits transference, coordination and integration of neurological information at sensorial, visual, and motor levels. It is thought to participate also in higher cognitive functions associated with language, abstract comprehension and social skills, like the ability of introspection [2]. Alterations of the CC include complete agenesis, partial agenesis and dysgenesis [3,4]. The real prevalence of agenesis of CC is estimated to be 0.3–0.7% [5] in the general population and 2–3% in the population with impaired neurological development.

Prenatal diagnosis of CC abnormalities is based on the identification of direct (non-visualization of the CC) or indirect signs (absent cavum septi pellucidi (CSP), abnormalities of the cerebral ventricles, widening of the interhemispheric fissure, alterations of the pericallosal artery, radial arrangement of cerebral sulci around the third ventricle) at the second or third trimester ultrasound scan [6,7].

Corpus callosum anomalies are often associated to other brain abnormalities, and magnetic resonance imaging (MRI) has proven to be useful in providing

CONTACT Nerea Maiz nmaiz@vhebron.net Department of Maternal-Fetal Medicine, Hospital Universitari Vall d'Hebron, Passeig Vall d'Hebron 119-129, Barcelona, Spain

additional information about associated anomalies like abnormal gyral patterns and heterotopias [8].

Previous studies reported a worse postnatal neurologic prognosis in the case of partial agenesis of the corpus callosum (ACC) compared to complete ACC [9]. Conversely, the latest meta-analysis published showed no difference in postnatal prognosis between partial and complete ACC, probably due to the misdiagnosis in the type of CC anomaly in prenatal examinations [10]. Prenatal imaging techniques such as ultrasound or MRI play an important role when it comes to differentiating the type of anomaly in order to give the parents accurate information about the neurological prognosis of the future child.

The main objective of our study was to evaluate the accuracy of prenatal ultrasound to diagnose CC anomalies compared to prenatal MRI, postnatal imaging techniques (ultrasound and MRI), and post-mortem examination in terminated pregnancies.

Materials and methods

This is an observational retrospective study on patients with a prenatal ultrasound diagnosis of abnormalities of the corpus callosum between January 2007 and December 2015 at the Maternal Fetal Medicine, Department at Vall d'Hebron Hospital in Barcelona, Spain. Ethical approval for this study was provided by the Comité de ética de investigación con medicamentos (CEIm) from the Vall d'Hebron University Hospital, Barcelona, Spain.

The study population was pregnancies with ultrasound diagnosis of fetal corpus callosum anomaly. We included consecutively women diagnosed at the routine second-trimester scan in our hospital or referred from other hospitals to the Fetal Medicine unit.

Information provided by prenatal ultrasound and MRI (abnormalities of the CC and associated cerebral alterations) and either the post-mortem examination or the postnatal ultrasound and MRI was collected for all fetuses and neonates. In all cases, a detailed fetal neurosonography was performed following ISUOG guidelines [11]. Corpus callosum anomalies were classified as complete ACC (absence of all components), partial ACC (absence of at least one region of the CC, with a short remnant always present), and dysgenesis of CC (Dysgenesis of corpus callosum was defined as a present but malformed corpus callosum, including anomalies in shape, thickness or length (all parts present)) [4]. All the scans were performed using transabdominal and transvaginal probes (when feasible) of 3–5 MHz and 10 MHz with Medison V20, Samsung WS80A Elite (Samsung Electronics Iberia SAU HME Health and Medical equipment, Seoul, South Korea) or Voluson E8 (GE Healthcare, Chicago, IL, USA) ultrasound equipment. All cerebral direct and indirect signs related to corpus callosum anomaly were evaluated. In the case of partial ACC and dysgenesis of CC, measurements of CC were compared with the existing standard reference charts [6,12]. When feasible, color mapping was used to visualize pericallosal arteries. A detailed study of the entire fetal anatomy was performed in all cases to rule out associated cerebral and extracerebral malformations.

In women who opted to continue with the pregnancy, a fetal MRI was scheduled in the third trimester, between 28 and 34 weeks. MRI studies were performed by a Pediatric Radiologist with high expertise in fetal brain imaging using a 1.5-T system (Avanto, Siemens, Erlangen, Germany) with high-speed sequences of T2 and T1-weighted (10–15 s). HASTE (Half-Fourier Acquired Single-Shot Turbo Spin-Echo) sequences were obtained, T2-weighted in a multiplanar fashion and T1-weighted on the axial plane. A post-mortem examination was offered in the case of termination of pregnancy (TOP).

Postnatal transfontanellar ultrasound and MRI were compared, when available, with prenatal imaging findings.

Statistical analysis

For the descriptive statistics, continuous variables are reported as median and range, whereas categorical variables are reported as absolute values and percentages. Kappa index was used to study the agreement in the type of CC anomaly between ultrasound and MRI, between ultrasound and post-mortem examination, and between MRI and post-mortem examination. A κ-index of 1 denotes perfect agreement and a κ-index of 0 denotes an agreement no better than that obtained at random. A κ-index value of 0.00–0.20 was considered as "poor," 0.21–0.40 "fair,", 0.41–0.60 "moderate," 0.61–0.80 "substantial," and 0.81–1.00 "near perfect" [13]. Statistical significance was fixed at $p < .05$. Statistical analysis was performed using IBM SPSS 23 Software (IBM SPSS Statistics for Windows, version 23.0.; IBM Corp, Armonk, NY).

Results

Eighty-six fetuses with callosal abnormalities were identified by prenatal ultrasound between 2007 and 2015. Median maternal age was 33 years (interquartile

range: 30–36). Regarding the type of conception, 82 (95%) were spontaneous pregnancies and four (5%) were obtained by assisted reproduction techniques (three cases of IVF and 1 ICSI). Seventy-nine (92%) were single gestations, while seven (8%) were multiple pregnancies (four dichorionic diamniotic and three monochorionic diamniotic sets of twins). Three (3%) cases were diagnosed in our hospital at the routine second-trimester ultrasound scan, and 83 (97%) were referred from other centers.

Forty-seven (55%) cases were diagnosed before 22 weeks while the other 39 (45%) were diagnosed after 22 weeks. Sixty (70.0%) cases were classified as complete ACC, 14 (16.3%) as partial ACC, and 12 (14%) as dysgenesis of CC. The alteration of CC was isolated in 42 cases (48.8%): 32 complete ACC (76.2%), six partial ACC (14.3%), and four dysgenesis of CC (9.5%). Prenatal ultrasound found other associated malformations in 44 cases (51.2%): 18 (40.9%) had other intracranial findings, 22 (50.0%) had additional extracranial findings, and four (9.1%) had both intracranial and extracranial findings. Intracranial findings were three cases of interhemispheric cyst, one case of holoprosencephaly, five cases of severe ventriculomegaly (more than 15 mm), six cases of posterior fossa abnormalities, five cases of neuronal migration disorders, one case of lipoma, and one case of arachnoid cyst. Extracranial findings included 11 cases of multiple malformations, two renal abnormalities, one goitre, five congenital heart defects, two genital abnormalities, one neural tube defect, two facial abnormalities, one bilateral talipes, and one case of diaphragmatic hernia. In the non-isolated cases, 28 (63.6%) had a complete ACC, eight (18.2%) partial ACC, and eight (18.2%) dysgenesis of CC.

Outcome

Two (2.3%) cases were lost to follow up. Fifty-eight (67.4%) cases opted for a TOP, 56 in singleton pregnancies and two as a selective termination in twin pregnancies. In 28 of the 58 (48.3%) cases the termination was carried out before 22 weeks and in 30 (51.7%) cases after 22 weeks. Twenty-six cases (30.2%) opted to continue with the pregnancy, from these, 2 (7.7%) cases resulted in a stillbirth and 24 (92.3%) were live births.

MRI

Fetal MRI was performed in 46 cases (53%). Fetal MRI found an additional intracranial abnormalities in seven of the 46 cases (15.2%): one case of hypothalamic hamartoma, one case of septo-optic dysplasia, one case of neuronal heterotopia, one case of white matter atrophy, one case of cingulate gyrus agenesis, and two cases of abnormal gyral pattern. Five of the seven cases had complete ACC, and two cases had dysgenesis of the CC.

Comparison between ultrasound and MRI

CC abnormality was confirmed in all 46 cases that had MRI. The agreement between prenatal ultrasound and MRI diagnoses regarding different subgroups of CC alteration is reported in Table 1. The kappa index is 0.86 (95% CI: 0.71–1.00).

Comparison between prenatal ultrasound and post-mortem examination

Fifty-four post-mortem examinations were authorized, 48 (88.9%) of which brought to diagnosis while the remaining 6 (11.1%) were not conclusive. In 46 (95.8%) of the 48 autopsies, a CC abnormality was ascertained, and in two (4.2%) cases, the CC was considered to be normal. Accuracy of prenatal ultrasound in differentiating different subgroups of CC abnormalities compared to post-mortem diagnosis is summarized in Table 1. Kappa index is 0.88 (95% CI: 0.75–1.00).

Comparison between prenatal MRI and post-mortem examination

In 23 cases where prenatal MRI had been performed, post-mortem examination was authorized. In three cases (13%), the post-mortem examination found no conclusive result, while in the remaining 20 cases (87%), CC abnormality was confirmed. The agreement between prenatal MRI and post-mortem examination is summarized in Table 2. Kappa index is 0.90 (95% CI: 0.73–1.00).

Comparison between prenatal ultrasound and postnatal ultrasound

Postnatal ultrasound data are available for 10 cases (41.7%) of the 24 newborns: eight cases of complete ACC and two cases of partial ACC. The agreement between prenatal and postnatal ultrasound regarding different subgroups of CC alteration is reported in Table 1. Kappa index is 0.75 (95% CI: 0.32–1.00).

Table 1. Prenatal ultrasound versus prenatal MRI, post-mortem examination and postnatal ultrasound.

Diagnosis	Prenatal MRI				Post-mortem examination[a]				Postnatal ultrasound			
Prenatal ultrasound	Complete ACC	Partial ACC	Dysgenesis CC	Total	Complete ACC	Partial ACC	Dysgenesis CC	Normal	Total	Complete ACC	Partial ACC	Dysgenesis CC	Total
Complete ACC	31	0	1	32	32	0	0	0	32	7	0	1	8
Partial ACC	1	7	0	8	0	6	0	0	6	0	2	0	2
Dysgenesis CC	0	1	5	6	0	1	7	2	10	0	0	0	0
Total	32	8	6	46	32	7	7	2	48	7	2	1	10
Kappa-index	0.86 (95% CI: 0.71–1.00)				0.88 (95% CI: 0.75–1.00)					0.75 (95% CI: 0.32–1.00)			

MRI: magnetic resonance imaging; ACC: agenesis of the corpus callosum; CC: corpus callosum; CI: confidence interval.
[a]Cases with data not available due to alive newborn, examination not informative, not accepted or lost to follow up were excluded.

Comparison between prenatal MRI and postnatal MRI

Postnatal MRI was performed on nine (37.5%) of the 24 livebirths. Accuracy in the diagnosis of different CC abnormality subgroups comparing prenatal and postnatal MRI is described in Table 2. Kappa index is 0.67 (95% CI: 0.24–1.00).

Discussion

Main findings

Our study confirms that prenatal ultrasound is a reliable tool for the diagnosis of corpus callosum abnormalities, given the excellent agreement with the results obtained by other diagnostic tools such as prenatal MRI and post-mortem examination. Similarly, there is a high match between prenatal MRI and post-mortem examination. Prenatal MRI detected additional brain abnormalities in 15% of cases.

Comparison to previous studies

MRI is considered the prenatal most accurate technique to diagnose subtle cerebral alterations associated with ACC [14,15]. A recent meta-analysis has shown MRI to perform better than the US, finding additional cerebral abnormalities in 7.8% of complete ACC and 11.8% of partial ACC [10]. Our study, with 15% of additional cerebral anomalies detected by fetal MRI, seems to confirm this finding. Cortical dysplasia is the most frequent cerebral anomaly associated with ACC, with a risk 7-fold higher in complete ACC when compared to partial ACC or CC hypoplasia. The most frequent type of cortical alteration was polymicrogyria [16]. Likewise, in our study, cortical anomalies were the most frequent associated findings in MRI.

Few studies have investigated the accuracy of prenatal and postnatal ultrasound and MRI in the diagnosis of the type of CC anomaly. Moreover, confirmation by post-mortem examination is often lacking or incomplete [17,18]. For this reason, we are convinced that it is very important to define the performance of prenatal and postnatal techniques in the diagnosis of the types of CC anomaly, in order to improve the accuracy of postnatal prognosis.

Limitations of the study

Our study has some limitations. MRI was performed only on 53% of our sample, owing to the gradual implementation of MRI in prenatal imaging and to a

Table 2. Prenatal MRI versus post-mortem examination and Postnatal MRI.

Diagnosis	Post-mortem examination[a]				Postnatal MRI				
Prenatal MRI	Complete ACC	Partial ACC	Dysgenesis CC	Total	Complete ACC	Partial ACC	Dysgenesis CC	Not performed	Total
Complete ACC	13	0	0	13	4	0	0	12	16
Partial ACC	0	2	1	3	0	1	1	1	3
Dysgenesis CC	0	0	4	4	0	0	0	1	1
Not realized	0	0	0	0	3	0	0	1	4
Total	13	2	5	20	7	1	1	15	24
Kappa-index	0.90 (95% CI: 0.73–1.00)				0.67 (95% CI: 0.24–1.00)				

MRI: magnetic resonance imaging; ACC: agenesis of the corpus callosum; CC: corpus callosum; CI: confidence interval.
[a]Cases with data not available due to alive newborn, examination not informative, not accepted or lost to follow up were excluded.

high number of patients opting for a TOP before MRI could be performed, between 28 and 34 weeks of gestation.

Literature is not consistent in the definition of the term dysgenesis of CC: some authors include partial ACC and CC hypoplasia in this definition [4], while others refer to dysgenesis as a CC alteration different from partial ACC [10]. In this study, we decided to classify CC anomalies in three groups (complete ACC, partial ACC, dysgenesis of CC) instead of two groups (complete ACC, dysgenesis CC including partial CC) in order to give more detailed information. We acknowledge that this detail would not make sense if the prognosis of partial ACC and dysgenesis of CC were the same. However, it is very premature to reach this conclusion. Nowadays data suggest that there is a difference in the prognosis of complete and partial ACC, and we do not know if future studies will find differences between partial ACC and other types of dysgenesis of CC. The percentage of TOP in our study (67.4%) is consistent with the literature [9,19]. However, it has to be pointed out that a large number of TOP, 28 cases (48%), took place before 22 weeks. It has to be stressed that in Spain termination of pregnancy is allowed by law before the 23rd week of gestation in the case of risk of severe fetal anomalies, and only since 2010 the terms for TOP have been extended beyond this limit in case of extremely severe and incurable abnormalities [20]. This change in the law permitted women to postpone the decision regarding the continuity of the pregnancy to the third trimester, depending on the findings of additional anomalies, while before 2010, many women would not want to take the risk and would terminate the pregnancy before 22 weeks.

Clinical relevance of the findings

There are two relevant findings in this study. First, it confirms the accuracy of ultrasound in the diagnosis of corpus callosum anomalies. Second, data support the recommendation to perform third-trimester MRI in order to detect additional intracranial anomalies that may alter the prognosis, thus allowing a more accurate counseling and more informed decision of parents about the continuation of the pregnancy.

Conclusion

Corpus callosum agenesis can be detected on the routine mid-trimester ultrasound scan. Prenatal ultrasound and MRI can accurately classify the type of CC abnormality. Moreover, third trimester MRI may detect additional intracranial anomalies in 15% of cases.

Disclosure statement

No potential conflict of interest was reported by the authors.

ORCID

Maddalena Santirocco http://orcid.org/0000-0001-6794-9120
Carlota Rodó http://orcid.org/0000-0001-8801-5677
Nerea Maiz http://orcid.org/0000-0001-8261-6383
Elena Carreras http://orcid.org/0000-0003-3471-7248

References

[1] Aboitiz F, Montiel J. One hundred million years of interhemispheric communication: the history of the corpus callosum. Braz J Med Biol Res. 2003;36(4):409–420.
[2] Paul LK, Brown WS, Adolphs R, et al. Agenesis of the corpus callosum: genetic, developmental and functional aspects of connectivity. Nat Rev Neurosci. 2007;8(4):287–299.
[3] Santo S, D'Antonio F, Homfray T, et al. Counseling in fetal medicine: agenesis of the corpus callosum. Ultrasound Obstet Gynecol. 2012;40(5):513–521.
[4] Palmer EE, Mowat D. Agenesis of the corpus callosum: a clinical approach to diagnosis. Am J Med Genet C. 2014;166C(2):184–197.
[5] Jeret JS, Serur D, Wisniewski K, et al. Frequency of agenesis of the corpus callosum in the

developmentally disabled population as determined by computerized tomography. Pediatr Neurosci. 1985–1986;12(2):101–103.

[6] Achiron R, Achiron A. Development of the human fetal corpus callosum: a high-resolution, cross-sectional sonographic study. Ultrasound Obstet Gynecol. 2001;18(4):343–347.

[7] Pilu G, Sandri F, Perolo A, et al. Sonography of fetal agenesis of the corpus callosum: a survey of 35 cases. Ultrasound Obstet Gynecol. 1993;3(5):318–329.

[8] Glenn OA, Goldstein RB, Li KC, et al. Fetal magnetic resonance imaging in the evaluation of fetuses referred for sonographically suspected abnormalities of the corpus callosum. J Ultrasound Med. 2005;24(6):791–804.

[9] Sotiriadis A, Makrydimas G. Neurodevelopment after prenatal diagnosis of isolated agenesis of the corpus callosum: an integrative review. Am J Obstet Gynecol. 2012;206(4):337.e1–337.e5.

[10] D'Antonio F, Pagani G, Familiari A, et al. Outcomes associated with isolated agenesis of the corpus callosum: a meta-analysis. Pediatrics. 2016;138(3). e20160445.

[11] International Society of Ultrasound in Obstetrics & Gynecology Education Committee. Sonographic examination of the fetal central nervous system: guidelines for performing the 'basic examination'. Ultrasound Obstet Gynecol. 2007;29(1):109–116.

[12] Malinger G, Zakut H. The corpus callosum: normal fetal development as shown by transvaginal sonography. Am J Roentgenol. 1993;161(5):1041–1043.

[13] Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33(1):159–174.

[14] Rossi AC, Prefumo F. Additional value of fetal magnetic resonance imaging in the prenatal diagnosis of central nervous system anomalies: a systematic review of the literature. Ultrasound Obstet Gynecol. 2014;44(4):388–393.

[15] Hetts SW, Sherr EH, Chao S, et al. Anomalies of the corpus callosum: an MR analysis of the phenotypic spectrum of associated malformations. Am J Roentgenol. 2006;187(5):1343–1348.

[16] Manganaro L, Bernardo S, De Vito C, et al. Role of fetal MRI in the evaluation of isolated and non-isolated corpus callosum dysgenesis: results of a cross-sectional study. Prenat Diagn. 2017;37(3):244–252.

[17] Craven I, Bradburn MJ, Griffiths PD. Antenatal diagnosis of agenesis of the corpus callosum. Clin Radiol. 2015;70(3):248–253.

[18] Rüland AM, Berg C, Gembruch U, et al. Prenatal diagnosis of anomalies of the corpus callosum over a 13-year period. Ultraschall Med. 2016;37(6):598–603.

[19] Ramelli G, Zanda N, Wyttenbach M, et al. The prognosis of agenesis of the corpus callosum might mostly be favourable. Swiss Med Wkly. 2006;136(25–26):404–405.

[20] Ley Orgánica 2/2010, de 3 de marzo, de salud sexual y reproductiva y de la interrupción voluntaria del embarazo [Organic Law 2/2010, of the 3rd of March, on Sexual and Reproductive Health and on the Voluntary Termination of Pregnancy]. BOE-A-2010-3514. 3514; 2010.