# COST-EFFECTIVENESS COMPARISON BETWEEN aCGH 60K PLATFORM AND aCGH 180K PLATFORM: RETROSPECTIVE ANALYSIS OF DIAGNOSTIC TECHNIQUES FOR NEURODEVELOPMENTAL DISORDERS AND CONGENITAL MALFORMATIONS CAUSED BY A GENETIC ALTERATION

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## 1. ABREVIATIONS

- aCGH: Array comparative genome hybridization
- ACMG: American College of Medical Genetics
- ADHD: Attention deficit and hyperactivity disorder
- ASD: Autism spectrum disorder
- BMD: Becker muscular dystrophy
- CMT1A: Charcot-Marie-Tooth Disease Type 1A
- CNV: Copy number variation
- DD: Developmental delay
- DGS: DiGeorge syndrome
- DMD: Duchenne muscular dystrophy
- FISH: Fluorescent *in situ* hybridization
- GDD: Global developmental delay
- GRC: Genome Reference Consortium
- ID: Intellectual disability
- ISCA: International Standard Cytogenetic Array
- ISCN: International System for Chromosome Nomenclature
- KS: Klinefelter syndrome
- MLPA: Multiplex ligation dependent probe amplification
- NDD: Neurodevelopmental disorder
- NGS: Next Generation Sequencing
- OCD: Obsessive-compulsive disorder
- OMIM: Online Mendelian Inheritance in Man
- SMS: Smith-Magenis syndrome
- TS: Turner syndrome
- VCFS: Velocardiofacial syndrome
- VOUS: Variants of unknown significance
- WBS: Williams-Beuren syndrome

## 2. ABSTRACT

Demonstrate that the array CGH 60K platform presents the best rate cost-effective for the genetic diagnosis in patients with neurodevelopmental disorders.

A retrospective analysis of the results from the array CGH technique 60K and 180K for prenatal and postnatal patients between 2016 and 2023 at the Hospital Universitari Sant Joan de Reus has been carried out. From all the 215 tests performed with the array CGH 180K, except of 2, could have been done with the array CGH 60K.

The implantation of the array CGH 60K platform in the diagnosis of neurodevelopmental disorders supposes a benefit in terms of cost-effectiveness.

# 3. BACKGROUND

## 3.1. ADVANCEMENTS IN GENETIC DIAGNOSIS

**Genetic diagnosis is a tool that allows us to know the presence of genetic alterations in an individual affected by a disease, presumably genetic, and to associate that genotype with a specific phenotype, as well as to predict the clinical evolution and prognosis of the individual** (Querejata MA, et al. 2012). Neurodevelopmental disorders (NDD), such as autism spectrum disorders (ASD) and intellectual disability (ID), whose etiology may be conditioned by genetic alterations, present a wide spectrum of overlapping clinical manifestations. The NDDs are characterized by presenting a high level of genetic heterogeneity that requires a comprehensive approach with the combination of genetic tests potentially necessary for each patient.

Many families of patients with suspected genetic syndromes become frustrated after many years of testing that fails to lead to a diagnosis. Although a new diagnosis does not necessarily influence treatment or outcome, it is of vital significance to many families. A specific diagnosis, such as developmental delay (DD) or dimorphic features, often facilitates getting services at school or through insurance carriers, allows physicians and genetic counselors to provide accurate recurrence risks for the couples and provide risks for the extended pedigree and, finally, allows the medical emphasis to progress from a diagnostic phase to an evaluation and long-term management phase (Darilek S, et al. 2008).

For decades, cytogenetic analysis of conventional karyotyping, also known as G-banded metaphase chromosomes, was the gold standard for ascertaining chromosomal abnormalities and the study of the five chromosomes involved in the most frequent aneuploidies (13, 18, 21, X and Y). Nonetheless, it is not sensitive enough to detect subtle chromosome rearrangements, of less than 4 Mb, due to the detection rate of the conventional karyotyping is deletions and duplications that are 5 – 10 Mb in size, the microdeletions (submicroscopic losses) and the microduplications (submicroscopic gains) are undetectable by conventional karyotyping. The introduction of FISH (fluorescent *in situ* hybridization) and MLPA (Multiplex Ligation dependent Probe Amplification) improved the diagnostic resolution to 40 – 250 Kb and has been considered an alternative for detecting submicroscopic chromosomal imbalances and rearrangements. Nevertheless, this type of analysis is a specialized method that requires prior knowledge of the chromosomal region of interest. (Cheung SW, et al. 2018; Levy B, et al. 2018; Rubio C, et al. 2013).

**The incorporation of genomic technologies, specifically the comparative genomic hybridization microarrays, has meant an important change in the routine of genetic diagnosis, being highly useful in cytogenetic diagnoses aimed at the detection and analysis of chromosomopathies that lead to gain or loss of genetic material.** It is an evolving technology for the rapid multiplex

detection of genomic imbalances that has been available for postnatal evaluation and diagnosis of individuals suspected of having a genetic condition. The multiplex format of the test allows simultaneous evaluation of multiple genetic disease specific regions, resulting in a more efficient consideration of possible diagnoses and cost savings over ordering testing of each locus individually, with the addition of clones or oligonucleotides providing backbone genomic coverage, having an advantage in terms of sensitivity, cost effectiveness, and higher resolution when compared with a conventional karyotype (Querejata MA, et al. 2012; Darilek S, et al. 2008).

### 3.2. ARRAY COMPARATIVE GENOME HYBRIDIZATION

**Array CGH** (Comparative Genome Hybridization) has become an essential routine diagnostic tool replacing conventional cytogenetic methods and has been recognized as a first-tier clinical diagnostic test for patients with developmental disabilities or congenital abnormalities without an obvious syndromic pattern (Miller DT, et al. 2010). When applied to array CGH, the most common clinical indications for testing are developmental delay and dimorphic features in a patient who does not demonstrate clinical findings that are immediately suggestive of a particular disorder. **Array CGH have enabled us to perform a genome-wide CNV (Copy Number Variation) analysis, detecting the gain or loss of genetic material, being useful for molecular diagnosis of genetic disorders.** Array CGH is used as the standard procedure for molecular cytogenetic analysis, and it allows the detection not only of aneuploidies, but also of all known microdeletion and microduplication disorders, including the detection of various submicroscopic genomic rearrangements involving exons or enhancers of disease-associated genes (Fukami M, et al. 2017; Darilek S, et al. 2008).

As advantages over conventional karyotyping, the genomic clones or oligonucleotides contained in an array CGH generally span most regions that are subject to recurrent deletions and duplications, allowing a greater resolution in the detection of CNV, going from an approximate resolution of 5 – 10 Mb in conventional karyotyping to several Kb in array CGH. Therefore, array CGH containing large-insert genomic clones and oligonucleotides distributed throughout the genome, have the potential to detect novel gains or losses that can then be correlated with a clinical phenotype (Querejata MA, et al. 2012; Darilek S, et al. 2008). Furthermore, the array allows these CNVs to be characterized in a precise way, knowing in detail the exact location of the alteration, its limits, size and its gene content, which results in a more effective genetic diagnosis and a more precise prediction of the disease associated with the genetic alteration. The detection rate for CNV among these patients has an average yield of 12,2% as compared to 3% for G-banded chromosome analysis. Therefore, array CGH has higher resolution than conventional G-banding karyotyping, although array CGH cannot detect copy-number neutral

translocations and inversions (Fukami M, et al. 2017). In addition, it does not require dividing cells to produce an analyzable result, therefore a faster technique, similar in response to aneuploidy analysis techniques (Querejata MA, et al. 2012; Darilek S, et al. 2008). In summary, it is a global genome analysis system at an adequate resolution where the technical requirements and test reactions are automatable and scalable, allowing an analysis with less bias than the conventional karyotype, reducing the subjectivity of the interpretation of the results, considering this technique a high-throughput strategy, and allowing to obtain multiple information in a short period of time (Cigudosa García, et al. 2012).

The main disadvantages of array CGH are the impossibility of detecting balanced rearrangements that do not suppose gain or loss of genetic material, such as translocations or inversions in chromosomes, which the conventional G-banding karyotyping can detect, and due to the increase in resolution, the appearance of unclear alterations, without apparent clinical explanation, known as uncertain significance (Querejata MA, et al. 2012). Also, aCGH technique is unable to detect if there is different clonal populations that may be present in a same sample, such as mosaicisms. It is impossible to detect polyploidies, because are global changes in the genetic material, undetectable with probe normalization systems. Even though, when using DNA it is not need living cells to establish cell cultures, the quantity of DNA needed to do an aCGH is higher than the needed in other techniques, additionally, this DNA sample must have an exceptional quality in order to get analyzable results. In addition, the array CGH technique can detect a high number of CNVs without a clear clinical impact, which difficult the interpretation of the results time (Cigudosa García, et al. 2012).

The American College of Medical Genetics (ACMG) recommends **array CGH as a first-tier diagnostic test for patients with an idiopathic developmental delay, intellectual disability, autism spectrum disorders or multiple congenital abnormalities** (Cheung SW, et al. 2018). Identification of such molecular defects is beneficial for patients because molecular diagnosis often allows better clinical management and accurate genetic counseling. Furthermore, identification of the genetic causes of genetic disorders provides advances in medical research (Fukami M, et al. 2017).

Microarray-based comparative genomic hybridization compares a patient's DNA sample to a normal control DNA sample to identify areas that are either over-represented or under-represented in the patient sample. In the aCGH approach, the patient's DNA and control DNA samples are cut into fragments then labeled with different fluorescent colors, usually, the patient's DNA sample is marked with a red colored fluorochrome (Cy5), and control DNA sample is marked with a green colored fluorochrome (Cy3). They are mixed together in equal proportions and placed into an array, which is typically a glass slide, containing multiple probes from representative sequences from across the human genome. The DNA mixture hybridizes in a competitive manner to complimentary sequences located

within the probe DNA on the array. The fluorescence intensity of every probe is measured using digital imaging software (Levy B, et al. 2018; Repáraz A, et al. 2018). This alternative is more beneficial, in terms of time and money, than performing a conventional karyotype in addition to two complementary locus-specific diagnoses, such as FISH and MLPA techniques. For practical purposes of detecting genetic alterations, array CGH increases the ability to detect genetic alterations by approximately 10% of the cases with dimorphic features, neurodevelopmental delay and normal karyotype (Cigudosa García, et al. 2012).

After a normalization process, a ratio of the fluorescence intensities between the patient sample and the normal control sample is calculated. Therefore, the resulting ratio of fluorescence intensity is proportional to the ratio of the copy numbers of the regions studied in the assay and the reference genomes. A ratio of one (1:1) indicates equal contributions from the patient and control sample which in turn represents a normal copy number at that locus. The resulting color will be the mixture of the two fluorophores, when Cy5 (red) and Cy3 (green) are used, the resulting color for the array is yellow. Array CGH log2-based intensity ratios provide useful information about genome-wide copy number alterations. The normal DNA copy number is two for all the autosomes. In an ideal situation, the normal DNA would correspond to a log2 ratio of 0 (log2 ratio 2/2). The log2 intensity ratios of a single copy loss would be -1 (log2 ratio 1/2), and a single copy gain would be 0.58 (log2 ratio 3/2). The goal is to effectively identify locations of gains or losses of DNA copy number. Using bioinformatics software, the color intensity of the image is translated into numerical data (log2 ratio) for each of the probes in the array (Miller DT, et al. 2010; Cigudosa García, et al. 2012; Repáraz A, et al. 2018).

**When a CNV is found, it should be classified as pathogenic if it is related to a pathology, as benign if it is clear that it does not have an influence on the phenotype, or as variants of uncertain significance (VOUS).** For the nomenclature of the chromosome formulas of the microarrays, the recommendations of the ISCN (International System for Chromosome Nomenclature) are applied. A normal array result for a male will be *arr(1-22)x2,(X,Y)x1* and for a female it will be *arr(1-22,X)x2*. To describe copy number variations of specific chromosomal regions, it will be necessary to specify which version of the Genome Reference Consortium (GRC) genome they are referenced against (GRCh37 or GRCh38). The description should continue with the affected cytoband followed by the chromosomal coordinates in parentheses and the number of copies present (McGowan-Jordan J, et al. 2016; Repáraz A, et al. 2018).

The resolution and diagnostic capability of aCGH depends on the number and types of probes used and their distribution across the entire genome. Depending on the number of arrays that the aCGH has, these can be 60K (60.000 arrays), 180K (180.000 arrays), 400K (400.000 arrays), 630K (630.000 arrays), 1.4M

(1,400,000 arrays), etc. **The commercial designs most used in clinical diagnosis are usually: 8x60K based of 8 samples in the array with 60.000 arrays each and 4x180K based of 4 samples in the array with 180.000 arrays each** (Miller DT, et al. 2010; Cigudosa García, et al. 2012; Levy B, et al. 2018; Repáraz A, et al. 2018).

**These copy number imbalances do not always relate to a pathogenic phenotype, the size alone does not determine pathogenicity of a CNV. The medical relevance of DNA copy number imbalances relates to the functional impact of the microdeletion or microduplication, which is more likely to have a phenotypic effect when the region of imbalance occurs in critical genes or an important regulatory region.** Microdeletions and microduplications that involve clinically significant genomic regions are also associated with specific genetic syndromes, many of which have congenital abnormalities as part of the phenotype. **The challenge is to discern what is pathogenic and what simply a benign polymorphism.** VOUS are genetic changes that are not commonly seen in the population and thus have little or any clinical evidence available to assess their pathogenicity. VOUS may represent benign familial variants that produce no clinical features or may be rare deleterious changes resulting in a clinical phenotype. The size, gene content, and inheritance pattern can help to discern whether VOUS are more likely to be benign or pathogenic (Levy B, et al. 2018). In cases in which no CNV is detected or a CNV described as a common polymorphism in the healthy population is detected, without pathological implication, a normal result is reported. In the cases detection of a CNV described as pathological, a subsequent verification is carried out with other molecular techniques, such as FISH or MLPA. In cases where a CNV of uncertain significance is detected, means a lack of knowledge of its phenotypic effect, being a good procedure to perform a genomic study on the parents. In the case of parental inheritance, it is reported that CNV could probably be benign but that there was a risk of incomplete penetrance or variable expressivity (Querejata MA, et al. 2012). In general, a CNV that is inherited from a normal parent is less likely to be clinically relevant, however, emerging data shows that a growing number of CNVs have incomplete penetrance and/or variable expressivity. Many of the CNVs that fall into this category affect neurocognitive development, and some evidence suggests that a second hit or increased mutational burden plays a role in the observed phenotype (Levy B, et al. 2018).

**Incomplete penetrance and variable expressivity are a significant concern for the correct interpretation of genetic variation and of diagnosing genetic disease.** The same genetic variant found in different individuals can cause a range of diverse phenotypes, from no discernible clinical phenotype to severe disease, even among related individuals. **Such variants can be said to display incomplete penetrance, a binary phenomenon where the genotype either causes the expected clinical phenotype or it does not, or they can be said to display variable expressivity, in which the same genotype can cause a**

**wide range of clinical symptoms across a spectrum.** Both incomplete penetrance and variable expressivity are thought to be caused by a range of factors, including common variants, variants in regulatory regions, epigenetics, environmental factors and lifestyle. Achieving a mechanistic understanding of how incomplete penetrance and variable expressivity occur will help inform diagnostic and prognostic testing, clinical management, and accurate genetic counseling (Kingdom R, et al. 2022).

*Table 1. Comparison between aCGH 60K platform and aCGH 180K platform*

| | aCGH 60K | aCGH 180K |
|---|---|---|
| **Minimum number of probes in order to report the result** | 5 probes | 5 probes |
| **OMIM syndromes** | 308 OMIM syndromes | 308 OMIM syndromes |
| **Average resolution compared to conventional karyotype** | 10 times higher | 50 times higher |
| **Average resolution in the genome** | 300 – 350 Kb | 100 Kb |
| **Minimum resolution of syndromic regions** | 100 Kb | 75 Kb |
| **Minimum resolution in critical genes** | 50 Kb | 40 Kb |

### 3.2.1. ARRAY CGH 60K

**Array CGH 60K** is designed to test for multiple malformation syndromes, with or without intellectual disability. The technique simultaneously detects the presence or the absence of genetic and chromosomal alterations (duplications or deletions) throughout the genome with an average resolution 10 times higher than the resolution of conventional karyotyping. In order to inform a structural alteration (microduplication or microdeletion) it should be detected by a minimum of 5 probes by the aCGH 60K platform.

It analyses 308 OMIM syndromes, together with other genetic regions responsible for multiple disorders. It offers a minimum resolution of 100 Kb in syndromic regions and 50 Kb in critical genes. The rest of the genome is analyzed with an average resolution of 300 - 350kb (*NIMGenetics Genómica y Medicina*. Array CHG – KarioNIM®. Madrid, Spain).

### 3.2.2. ARRAY CGH 180K

**Array CGH 180K** has facilitated the diagnosis of single-gene disorders due to haploinsufficiency resulting from intragenic copy number change affecting even a single exon. Therefore, it is designed to test for neurodevelopmental disorders and multiple malformation syndromes. The technique has an average resolution 50 times higher than the resolution of conventional karyotyping. In order to report

a structural alteration (microduplication or microdeletion) it should be detected by a minimum of 5 probes by the aCGH 180K platform.

It analyses 308 OMIM syndromes, together with other genetic regions responsible for various disorders. It offers a minimum resolution of 75 Kb in syndromic regions and 40 Kb in critical genes. The rest of the genome is analyzed with an average resolution of 100 Kb.

In addition, there is an array CGH 180K specifically designed for the study of autism spectrum disorders. The critical regions affected with microdeletions or microduplications associated with susceptibility to ASD (syndromic or non-syndromic), involves a total 45 syndromes related with autism spectrum disorder.

The aim of the design is the study of neurodevelopmental disorders and polymalformation syndromes, with a powerful increase in the detection capacity, with a resolution of 15 Kb in 115 genes associated with ASD (*NIMGenetics Genómica y Medicina*. Array CHG – KarioNIM®. Madrid, Spain).

### 3.3. GENETIC DISORDERS DETECTED WITH ARRAY CGH

The use of array CGH is indicated in patients with a normal karyotype and a clinical picture with unexplained developmental delay, congenital anomalies, dimorphic features or clinical presentations that suggest a specific chromosomal syndrome. It is also indicated in altered karyotypes in patients with apparently balanced rearrangements with an abnormal clinical phenotype, in the presence of duplications or deletions in the karyotype to determine the limits of the altered region and when chromosome markers are identified, to determine their origin (*BioArray: Diagnóstico Genético.* Array CGH. Elche, Alicante, Spain).

The International Standard Cytogenetic Array (ISCA) consortium affirms that aCGH technique is potentially important in the prenatal diagnosis**. In postnatal studies of individuals with congenital abnormalities, developmental delay or intellectual disability, array CGH has an additional diagnostic rate between 12% and 15%, compared to the classic approach with conventional cytogenetics of about 3% to 4%**. (Levy B, et al. 2018). It is estimated that the prevalence in the general population of developmental delay and intellectual disability is about 1% to 3%, autism spectrum disorders is 0,7% and congenital malformations between 2% to 3%, representing a serious health and social problem (Castells Sarret N, et al. 2018).

**Developmental delay, intellectual disability and learning disorder are severe conditions and in general terms are characterized by the deterioration of cognitive abilities and adaptive skills.** These affections are considered important in the public health ambit and are considered important at a clinical level with an etiology that remains unknown in most cases. **The appearance of these conditions is influenced by genetic, environmental,**

**infectious and prenatal factors. In approximately half of the cases the final etiology cannot be identified. The results of several studies have shown that up to 40% of cases may have a genetic basis, including chromosomal abnormalities (4% - 28%), recognizable syndromes (3% - 7%) and known monogenic diseases (3% - 9%).** The proportion of cases in which a definitive cause is identified also varies according to the severity of the disorder, approximately 30% of several developmental delay and 70% of mild to low developmental delay remain without an etiological diagnosis. There is no general consensus on performing array CGH in patients with infertility, sterility and/or in women with repeated spontaneous abortions, but they could eventually benefit from this type of study. Furthermore, in patients with idiopathic short stature, studies guided at the *SHOX* and *PAR1* genes of the X chromosome are frequently performed to detect microdeletions or microduplications. Array CGH technique not only is performed to detect clinically recognizable genetic syndromes whose patients manifest developmental delay or intellectual disability, but instead, some diagnostic and research centers use customized array CGH applied to certain pathologies or a group of pathologies (Cigudosa García, et al. 2012).

### 3.4. OPTIMIZING COST-EFFECTIVE ARRAY CGH PLATFORMS FOR GENETIC ANALYSIS

**There is an evidence in both scientific and economic literature that demonstrates the high cost-effectiveness index by using array CGH in the diagnosis of language and learning disabilities.** This important advantage is due to the greater resolution and higher sensitivity of the technique, as mentioned previously. The effectiveness represents cost savings thanks to the reduction of the diagnostic tests necessary to reach a genetic diagnosis.

Even though neurodevelopmental disorders and intellectual disabilities have not a cure yet, an accurate diagnosis allows to know the syndrome or the genetic condition that causes the global developmental delay (GDD), and it is essential in order to define prognosis and allow the families an appropriate management, both clinical and social, as well as facilitates a genetic counselling and covers the educational needs of the patients (*NIMGenetics Genómica y Medicina*. Array CHG – KarioNIM®. Madrid, Spain). The cost for a pathological result of a conventional karyotype is 1.931€ and the cost for a pathological result of array CGH is 1.979€. Triple the number of diagnoses, that involves going from diagnosing 7% to 22% of patients, results on an increase of 1% in the cost per diagnosis. From this point of view, the replacement of cytogenetics by array CGH is justified for both scientific and healthcare reasons and is irrelevant from an economic point of view (Cigudosa García, et al. 2012).

The International Standard Cytogenetic Array (ISCA) consortium provides recommendations that have been adopted by most array manufacturers. **Due to**

**clinical and economic considerations, scientific guidelines recommend the use of arrays as a first-line diagnostic technique in patients with developmental delay/intellectual disability, congenital malformations and/or autism spectrum disorder, replacing karyotyping, MLPA and FISH techniques** (Castells Sarret N, et al. 2018).

## 4. HYPOTHESIS

By replacing the aCGH 180K platform for the aCGH 60K platform in the study of neurodevelopmental disorders caused by a genetic alteration, all or almost all the samples performed could have been resulted in the same diagnosis but lowering the price of the diagnostic tool, therefore could suppose a cost-effective strategy.

## 5. OBJECTIVES

The main aim of the study is to demonstrate that the array CGH 60K platform, compared to the array CGH 180K platform, presents a better rate cost-effective in terms of cost saving and efficiency for the genetic diagnosis.

Another secondary objective of the study include to evaluate the relation between the CNV found in the array CGH analysis of the patients and their respective clinical manifestations, to determine if the gain or the loss of genetic material causes the symptomatology, classifying the copy number variant as pathogenic, being or a variant of uncertain significance.

## 6. MATERIALS AND METHODS

An exhaustive review of the clinical history of the patients included in the study was carried on in order to evaluate the relation between phenotype-genotype allowing an accurate interpretation of the clinical results and a classification of the CNVs reported in the patients with genetic abnormalities. Some databases have been consulted for the appropriate interpretation of the CNVs, such as OMIM (Online Mendelian Inheritance in Man), ORPHANET and DECIPHER, and its respective classification into benign, pathogenic or uncertain significance. The results have been defined based on the recommendations from ISCA and ACMG.

In addition, a cost-effectiveness analysis has been done based on the proportion of the aCGH 60K and the aCGH 180K genetic analysis carried out, to determine the best platform in cost savings and efficiency for the genetic diagnosis of patients with neurodevelopmental disorders or congenital malformations suspected of genomic imbalances.

## 7. RESULTS AND DISUSSION

A retrospective analysis has been carried out based on the results obtained from the array CGH technique of both 60K and 180K for prenatal and postnatal patients in a period of time between 2016 and 2023 at the Hospital Universitari Sant Joan de Reus for the detection of associated CNVs to genetic alterations related to neurodevelopmental disorders.

*Table 2. Total samples and positive samples obtained with the 60K and 180K array CGH technique in abortive samples, amniotic fluid and blood*

| Samples | Nº | % |
|---|---|---|
| **Total samples** | **463** | **100%** |
| ARRAY CGH 60K (ABORTION) | 9 | 1,94% |
| ARRAY CGH 60K (PRENATAL) | 132 | 28,51% |
| ARRAY CGH 60K (POSTNATAL) | 107 | 23,11% |
| ARRAY CGH 180K (POSTNATAL) | 215 | 46,44% |
| **Positive samples** | **48** | **10,37%** |
| ARRAY CGH 60K (ABORTION) | 0 | 0% |
| ARRAY CGH 60K (PRENATAL) | 5 | 10,42% |
| ARRAY CGH 60K (POSTNATAL) | 15 | 31,25% |
| ARRAY CGH 180K (POSTNATAL) | 28 | 58,33% |

As can be seen in *Table 2*, we started from a total sample of 463 patients in this study who have undergone a comparative genomic hybridization analysis, of which 48 positive samples have been reported for microdeletions or microduplications in the genome, which establishes 10,37% of the analyzed population. This value consists of 5 positive samples with the prenatal 60K array CGH in amniotic fluid of the 132 analyzes performed, 15 positive samples with the postnatal 60K array CGH in blood of the 107 analyzes performed and 28 positive samples with the postnatal 180K CGH array in blood of the 215 analyzes performed. It should be noted that 60K array CGH have been performed on abortive samples, all of which have been negative.

*Table 3. Classification of the CNVs*

| CNV | Nº | % |
|---|---|---|
| **Benign** | **0** | **0%** |
| **Pathogenic** | **21** | **43,75%** |
| **Accidental discovery** | **1** | **2,08%** |
| **VOUS** | **26** | **54,17%** |
| VOUS likely benign | 1 | 3,85% |
| VOUS likely pathogenic | 16 | 61,54% |
| VOUS that do not explain the clinical context | 9 | 34,61% |

As shown in *Table 3*, out of the 48 positive samples for microdeletions or microduplications in the genome, no benign polymorphism has been reported and 21 pathogenic CNVs have been informed (43,75%). In addition, an accidental discovery has been found (2,08%). Furthermore, within the 26 results obtained classified as of uncertain significance (54,17%), there is one possibly benign

polymorphism (3,85%), 16 possibly pathogenic CNVs (61,54%) and 9 results that do not explain the clinical context of the patient (34,61%).

*Table 4. Phenotype-genotype associations of the CNVs*

| aCGH platform | Genetic affection | Described syndrome | Patient's clinical manifestations | Syndrome's clinical features related with nervous system |
|---|---|---|---|---|
| **Benign and pathogenic** | | | | |
| aCGH 180K | 1q21.1q21.2 x3 (2,9 Mb) | 1q21.1 microduplication syndrome | Global hypotonia with motor and language delay | Macrocephaly, NDD, ID, psychiatric disturbances and mild facial dimorphism |
| aCGH 60K | 2q24.2 x1 (2,37 Mb) | 2q24.2 microdeletion syndrome | Severe ASD | Seizures, microcephaly, dimorphic features, growth retardation, NDD and ASD |
| aCGH 180K | 2p25.3 x1 (2,23 Mb) | 2p25.3 deletion syndrome | GDD and ASD | NDD, ID, speech delay, ASD, aggression and stereotypic movements |
| aCGH 180K | 7q11.23 x1 (1,9 Mb) | Williams-Beuren syndrome | GDD | Mental retardation, relative sparing of language, poor visual-motor integration, poor visual-spatial construction, hypersensitivity to sound, hypotonia, hyperreflexia, poor balance, poor coordination, type I Chiari malformation, friendly personality, gregarious, cocktail party personality, strong attraction to music, anxiety, phobias, ADHD and OCD |
| aCGH 60K | 15q11.2 x1 (4,8 Mb) | Angelman syndrome | Irritability crisis and swallowing disorder, frequent axial bowing, generalized hypertonia and right occipital plagiocephaly | DD, severe mental retardation, absent speech, ataxia with jerky arm movements, wide-based gait, clumsiness, unsteadiness, tremor of limbs, hypotonia, seizures, hyperreflexia, characteristic arm position with wrist and elbow flexion, abnormal sleep-wake cycles, decreased need for sleep, characteristic electroencephalogram discharges, mild cortical atrophy |
| aCGH 180K | 16p11.2 x1 (1,6 Mb) | 16p11.2 microdeletion syndrome | GDD, behavior disorder and macrocephaly | Developmental and language delays, mild ID, social impairments and ASD |
| aCGH 60K | 16p11.2p12.2 x3 (7,7 Mb) | 16p11.2p12.2 microduplication syndrome | Behavior problems, growth retardation, celiac disease, left temporal gliosis, occipital epilepsy and moderate mental retardation | Developmental/psychomotor delay, ID, ASD and/or obsessive and repetitive behavior, behavioral problems and dimorphic facial features |
| aCGH 180K (3) and aCGH 60K (1) | 16p13.11 x1 (2,8 Mb (3) and 1,7 Mb (1))<br>**Described in 4 patients* | 16p13.11 microdeletion syndrome | Psychomotor delay, cognitive difficulties, ID, NDD, social impairments, learning disabilities, psychological problems, anxiety and depression<br>**3 are relatives (father/daughters)* | NDD, microcephaly, epilepsy, short stature, facial dimorphism and behavioral problems |
| aCGH 60K | 17q12 x3 (1,5 Mb) | 17q12 microduplication syndrome | Syndromic phenotype and affected by ASD | NDD, mild to severe ID, speech delay, seizures, microcephaly, behavioral abnormalities, ASD and schizophrenia |
| aCGH 180K | 17q12 x1 (1,4 Mb) | 17q12 microdeletion syndrome | Cognitive impairments, behavior problems and DD | NDD, cognitive impairment, DD, speech delay and ASD |
| aCGH 60K (1) and aCGH 180K (2) | 22q11.21 x1 (2 Mb (1) and 2,8 Mb (2))<br>**Described in 3 patients* | DiGeorge syndrome or Velocardiofacial syndrome | GDD and mild cognitive impairment<br>**One prenatal patient* | Mild to moderate learning difficulties, delayed psychomotor development, late-onset speech development, tetany, seizures, ADHD, schizophrenia, bipolar disorder |
| aCGH 60K | 22q12.3q13.3 x3 (13,2 Mb) | 22q12.3q13.3 *de novo* terminal duplication | Intrauterine growth retardation and suspected heart disease<br>**Prenatal patient* | NDD, delayed speech, language development delay, expressive language delay, ID, intrauterine growth retardation, proportionate short stature, hypertrophic cardiomyopathy and pulmonic steatosis |
| aCGH 60K | Xp21.1 x0 (394 Kb) | Duchenne-Becker muscular dystrophy | **Prenatal patient* | Cardiomyopathy, congestive heart failure, pulmonary hypoventilation, respiratory failure, increased lordosis, scoliosis, flexion contractures, calf muscle pseudohypertrophy, weakness, mental retardation, hypotonia, waddling gait and hyporeflexia, positive gowers sign |

| aCGH platform | Genetic affection | Described syndrome | Patient's clinical manifestations | Syndrome's clinical features related with nervous system |
|---|---|---|---|---|
| aCGH 60K | 47, XXY | Klinefelter syndrome | Hypertonia in the lower extremities and stagnant head circumference | Neurocognitive disabilities, neuropsychological difficulties, language and speech problems |
| aCGH 180K | 47,XXX | Triple X syndrome or trisomy X | Moderate/severe ASD, sleep disorder and eating disorder | NDD, speech-language developmental delay, motor developmental delay, hypotonia, developmental dyspraxia, cognitive deficits, learning disabilities, ADHD, anxiety, depression/dysthymia and psychotic disorders |
| aCGH 180K | Xp22.33p22.12 x1 (21,5 Mb) Xp22.12p11.1 x3 (36,7 Mb) 9p24.3p24.1 x3 (7,1 Mb) | - | Typical traits from Turner syndrome | Short stature and some somatic traits typical of Turner syndrome whereas gonadal function is generally preserved |
| **Accidental discovery** | | | | |
| aCGH 180K | 17p12 x3 (1,3 Mb) | Charcot-Marie-Tooth Disease Type 1A | Behavior disorder, language delay and hyperactivity | Distal limb muscle weakness due to peripheral neuropathy, distal limb muscle atrophy due to peripheral neuropathy, 'steppage' gait, foot drop, cold-induced muscle cramps, distal sensory impairment, hyporeflexia, areflexia, decreased motor nerve conduction velocity, hypertrophic nerve changes, 'onion bulb' formations seen on nerve biopsy, segmental demyelination/remyelination seen on nerve biopsy, decreased number of myelinated fibers and myelin outfoldings |
| **VOUS likely benign** | | | | |
| aCGH 180K | 6p12.3 x1 (580 Kb) Yq11.22 x3 (80 Kb) | - | ID, language delay and learning difficulties | - |
| **VOUS likely pathogenic** | | | | |
| aCGH 60K | 2p16.3 x1 (190 Kb) | 2p16.3 deletion syndrome | DD and ASD | ASD, schizophrenia, DD, ID and dimorphic features |
| aCGH 60K | 3q29 x1 (621 Kb) | 3q29 microdeletion syndrome | Behavior disorder, severe ID and familial paroxysmal ataxia | Mild-to-moderate ID, slightly dimorphic facial features, ASD and gait ataxia |
| aCGH 180K (2) | 4p15.32 x3 (970 Kb and 946 Kb) **Described in 2 patients* | 4p15.32 *de novo* interstitial duplication | ADHD, memory concentration and orientation disorders, suspicion of mild cognitive delay, DD and cognitive impairment | ID, GDD and ASD |
| aCGH 60K | 5p15.1p14.3 x3 (2 Mb) | Trisomy 5p | NDD and epilepsy | ASD and ID |
| aCGH 60K (2) | 8p23.1 x3 (525 Kb (2)) **Described in 2 patients* | 8p23.1 duplication syndrome | The fetus presents polyhydramnios and the mother doesn't express any symptomatology **One prenatal patient and both are relatives (mother/fetus)* | Mild to moderate DD, ID, mild facial dimorphism, macrocephaly, behavioral abnormalities and seizures |
| aCGH 60K | arr(9)x3 | Mosaic trisomy 9 | Thymic hypoplasia, silver hair, short limbs, scaphocephaly, dolichocephaly, flat nasal root, small sunken eyes, low-set ears, prominent forehead, underdeveloped genitalia and empty scrotal sacs | ID, growth delay, DD and facial dimorphism |
| aCGH 180K | 15q13.2q13.3 x3 (2,47 Mb) 22q11.23 x3 (1,32 Mb) | 15q13.3 microduplication syndrome | GDD, ASD with cognitive impairment, macrocephaly and ID | DD, ID, epilepsy, hypotonia, ASD, ADHD and schizophrenia |
| aCGH 60K | 16p12.2 x1 (448 Kb) | 16p12.2 microdeletion syndrome | ASD and epilepsy | DD, delayed speech, ID, macrocephaly, epilepsy, psychiatric and behavioral problems. |
| aCGH 60K | 16q23.2 x1 (419 Kb) | 16q23.2 *de novo* deletion | Cognitive impairment, language delay and epilepsy. | ADHD, ASD, delayed fine motor development, delayed speech and language development, GDD and ID |
| aCGH 180K | 17p11.2 x1 (300 Kb) | Smith-Magenis syndrome | ASD, speech delay and café-au-lait spots | Delayed speech and language development and GDD |

| aCGH platform | Genetic affection | Described syndrome | Patient's clinical manifestations | Syndrome's clinical features related with nervous system |
|---|---|---|---|---|
| aCGH 180K | 22q11.21 x3 (2,73 Mb) Xp22.33 / Yp11.32 x2 (340 Kb) | 22q11.2 duplication syndrome | Language delay, ID and hyperkinesia | Specific learning disability, GDD, dysplastic corpus callosum and ID |
| aCGH 60K (1) and aCGH 180K (2) | Xp22.31 x3 (1,5 Mb, 1,6 Mb and 204 Kb) *Described in 3 patients* | Xp22.31p22.2 duplication syndrome | ASD, learning disabilities, GDD, psychomotor delay, hypotonia and atrial septal defect *One is diagnosed with Kabuki syndrome by NGS* | DD, ID, learning and behavioral problems and mild dimorphic features |
| **VOUS that do not explain the clinical context** | | | | |
| aCGH 60K | 1q21.1q21.2 x4 (2 Mb) 5q21.2q21.3 x1 (1,3 Mb) | - | Intracardiac hyperechogenic focus and single umbilical artery *Prenatal patient* | - |
| aCGH 180K | 1q25.1 x1 (90 Kb) | - | ASD, GDD and hyperkinesia | - |
| aCGH 180K | 2q14.3 x3 (1,8 Mb) | - | GDD | - |
| aCGH 180K | 3p12.3 x1 (737 Kb) | - | GDD | - |
| aCGH 180K | 9p23 x3 (946 Kb) | - | GDD | - |
| aCGH 180K | 14q31.3 x3 (922 Kb) | - | Atrial septal defect, peculiar phenotype and dimorphic features suggestive from Rubinstein Taybi syndrome | - |
| aCGH 180K | 15q13.1 x3 (160 Kb) | - | ASD | - |
| aCGH 180K | 16q22.1 x1 (643 Kb) | - | GDD | - |
| aCGH 60K | Xq27.1 x2 (270 Kb) | - | ASD *Diagnosed with Wiedemann – Steiner syndrome by NGS* | - |

In *Table 4*, there is a compilation of all the 48 positive samples for microdeletions or microduplications in the genome, where the aCGH platform used to perform the analysis, the genetic affectation with the CNV size, the described syndrome, the patient's clinical manifestations and the syndrome's clinical features related with the nervous system are informed.

### 7.1. BENIGN AND PATHOGENIC CNVs

Considering the 48 positive samples from patients with microdeletions and microduplications, as mentioned before, no benign polymorphism has been reported and 21 CNVs are pathogenic and have been associated with the following described syndromes:

- 1q21.1 microduplication syndrome: 1q21.1 microduplication syndrome is a rare partial autosomal trisomy/tetrasomy with incomplete penetrance and variable expression characterized by macrocephaly, developmental delay, intellectual disability, psychiatric disturbances (autism spectrum disorder, attention deficit hyperactivity disorder, schizophrenia and mood disorders) and mild facial dimorphism (high forehead and hypertelorism). Other associated features include congenital heart defects, hypotonia, short stature and scoliosis (OMIM 612475). The prevalence is <1 / 1.000.000 and the inheritance is autosomal dominant (ORPHANET 250994). The patient in our study who presented the duplication in the chromosome 1 (1q21.1q21.2)

manifests the following symptomatology related to the 1q21.1 microduplication syndrome described in the bibliography: global hypotonia with motor and language delay. Therefore, we can affirm that the CNV is pathogenic.

- 2q24.2 microdeletion syndrome: 2q24 microdeletion syndrome is a chromosomal anomaly consisting of a partial long arm deletion of chromosome 2 and characterized clinically by a wide range of manifestations (depending on the specific region deleted) which can include seizures, microcephaly, dimorphic features, cleft palate, eye abnormalities (coloboma, cataract and microphthalmia), growth retardation, failure to thrive, heart defects, limb anomalies, developmental delay and autism spectrum disorder. The prevalence is <1 / 1.000.000 and the inheritance is unknown (ORPHANET 1617). In the DECIPHER database are reported three patients (249144, 285759 and 285787) with the deletion in the chromosome 2 with a similar size CNV (2,37 Mb). The first one presents the following phenotypes related to abnormalities of the nervous system: atypical behavior and intellectual disability. The other two have phenotypes related to congenital hypothyroidism, cyanosis, constipation, delayed speech and language development, generalized neonatal hypotonia, high-output congestive heart failure, hyperactivity, hypermetropia, hyperreflexia, hypoplastic toenails, irritability, motor delay, muscle weakness, respiratory failure, weight loss and pulmonary arterial hypertension. The patient in our study who presented the deletion in the chromosome 2 (2q24.2) manifests the following symptomatology related to the 2q24.2 microdeletion syndrome described in the bibliography: severe autism spectrum disorder. Therefore, we can affirm that the CNV is pathogenic.

- 2p25.3 deletion syndrome: 2p25.3 duplication syndrome is an intellectual developmental disorder-39 caused by a deletion in the *MYT1L* gene encoding myelin transcription factor 1-like on chromosome 2p25.3 and the inheritance is autosomal dominant. The clinical features are: obesity (in most patients), delayed development, intellectual disability, speech delay, autistic features, aggression and stereotypic movements (OMIM 616521). The patient in our study who presented the deletion in the chromosome 2 manifests the following symptomatology related to the 2p25.3 deletion syndrome described in the bibliography: global developmental delay and autism spectrum disorder. Therefore, we can affirm that the CNV is pathogenic.

- Williams-Beuren syndrome (WBS) is caused by a 1,5 to 1,8 Mb **heterozygous deletion on chromosome 7q11.23** and the inheritance is autosomal dominant. The clinical features are: short stature, abnormal weight gain, intrauterine growth retardation, medial eyebrow flare, flat midface, periorbital fullness (puffy eyes), epicanthal folds, long philtrum, sensorineural

hearing loss, mild to moderate, hyperacusis, phonophobia, abnormal brain auditory evoked responses, decreased or absent ipsilateral acoustic reflex response to maximum stimulation, stellate pattern of iris, strabismus, altered visual acuity, depressed nasal bridge, anteverted nares, thick lips, hypodontia, microdontia, supravalvular aortic stenosis, valvular aortic stenosis, bicuspid aortic valve, mitral valve prolapse, mitral regurgitation, coronary artery stenosis, pulmonary valve stenosis, atrial septal defect, ventricular septal defect, peripheral pulmonary artery stenosis, systemic hypertension, vocal cord paralysis, pectus excavatum, inguinal hernia, chronic constipation, diverticulosis, colic, difficulty feeding, gastroesophageal reflux, textured-food intolerance, small kidneys, solitary kidney, pelvic kidney, nephrocalcinosis, renal insufficiency, renal artery stenosis, vesicoureteral reflux, bladder diverticula, urethral stenosis, recurrent urinary tract infections, voiding frequency/urgency, enuresis, delayed toilet training, joint contractures, joint laxity, osteopenia, osteoporosis, kyphoscoliosis, joint limitation, hallux valgus, decreased skin stiffness, easier stretching, soft skin, increased wrinkles, abnormal scarring, less pressure required to lift skin, longer retraction time after stretching, decreased viscoelasticity, premature aging, mild, hypoplastic nails, premature graying, mental retardation (average IQ 56), relative sparing of language, poor visual-motor integration (range 41-80), poor visual-spatial construction, hypersensitivity to sound, hypotonia, hyperreflexia (particularly in lower extremities, more prevalent in adolescents and adults), poor balance, poor coordination, type I Chiari malformation, friendly personality, gregarious, cocktail party personality, strong attraction to music, anxiety, phobias, attention deficit and hyperactivity disorder (ADHD) obsessive-compulsive traits (OCD), harsh, brassy or hoarse voice, hyperkalemia, glucose intolerance, diabetes mellitus, early-onset puberty (menarche about 2 years early), hypothyroidism (subclinical), incidence 1 in 8,000 live births and main aspects of phenotype attributed to defects in *GTF2IRD1* and *GTF2I* genes (OMIM 194050). The Williams-Beuren syndrome has been diagnosed in a patient of the study who manifest the following clinical features related with the WBS: global development delay. As this is a well described genetic disorder, it can be stated that the CNV is pathogenic.

- Angelman syndrome is caused by a **deletion of chromosome 15q11.2 when the loss is from a maternal allele**, but instead, when the deletion of chromosome 15q11.2 in from a paternal allele the affection causes Prader Willi syndrome. In both cases the deletion affects the *UBE3A* gene and the inheritance is autosomal dominant. The clinical features are: Obesity, microcephaly postnatal, brachycephaly, flat occiput, occipital Groove, prognathia, strabismus most frequently exotropia, ocular hypopigmentation, refractive errors such as astigmatism, hyperopia and myopia, protruding tongue, macrostomia, excessive drooling, widely spaced teeth, feeding difficulties in neonatal period, excessive chewing/mouthing behaviors,

abnormal food-related behaviors, constipation, scoliosis, hypopigmentation, developmental delay, severe mental retardation, absent speech, ataxia with jerky arm movements, wide-based gait, clumsiness, unsteadiness, tremor of limbs, hypotonia, seizures, hyperreflexia, characteristic arm position with wrist and elbow flexion, abnormal sleep-wake cycles, decreased need for sleep, characteristic electroencephalogram discharges, mild cortical atrophy on CT or MRI, paroxysmal laughter, easily excitable, attraction to/fascination with water, crinkly items such as paper and plastic, imprinted disorder, onset between 6 and 12 months of age, increased sensitivity to heat, incidence of 1 in 10,000 to 1 in 20,000, 70% due to de novo maternal deletion of 15q11.2-q13, 2% due to paternal uniparental disomy of 15q11.2-q13 and 2-3% due to imprinting defects (OMIM 105830). The Angelman syndrome has been diagnosed in a patient of the study who manifest the following clinical features related with the syndrome previously mentioned: irritability crisis and swallowing disorder, frequent axial bowing, generalized hypertonia and right occipital plagiocephaly. As this is a well described genetic disorder, it can be stated that the CNV is pathogenic.

- 16p11.2 microdeletion syndrome: 16p11.2 microdeletion syndrome is a rare chromosomal anomaly syndrome resulting from the partial deletion of the short arm of chromosome 16 with a highly variable phenotype typically characterized by developmental and language delays, mild intellectual disability, social impairments and autism spectrum disorder. Macrocephaly (apparent by 2 years of age), structural brain malformations, epilepsy, vertebral anomalies and predisposition to obesity are frequently associated (OMIM 613444). The prevalence is 1-5 / 10.000 and the inheritance is autosomal dominant (ORPHANET 261222). The patient in our study who presented the deletion in the chromosome 16 manifests the following symptomatology related to the 16p11.2 deletion syndrome described in the bibliography: global developmental delay, behavior disorder and macrocephaly. Therefore, we can affirm that the CNV is pathogenic.

- 16p11.2p12.2 microduplication syndrome: 16p11.2p12.2 microduplication syndrome is a rare chromosomal anomaly syndrome resulting from the partial duplication of the short arm of chromosome 16 with a highly variable phenotype typically characterized by developmental/psychomotor delay (particularly of speech), intellectual disability, autism spectrum disorder and/or obsessive and repetitive behavior, behavioral problems (aggression and outbursts), dimorphic facial features (triangular face, deep set eyes, broad, prominent nasal bridge, upslanting or narrow palpebral features and hypertelorism). Additionally, finger/hand anomalies, short stature, microcephaly and slender build are frequently described. The prevalence is <1 / 1.000.000 and the inheritance is unknown (ORPHANET 261204). The patient that presents the duplication on the chromosome 16 expresses the

following symptomatology related to the 16p11.2p12.2 microduplication syndrome described in the bibliography: behavior problems, growth retardation, celiac disease, left temporal gliosis, occipital epilepsy, moderate mental retardation and peculiar phenotype. Therefore, we can affirm that the CNV is pathogenic.

- 16p13.11 microdeletion syndrome: The 16p13.11 deletion syndrome has been diagnosed in 4 patients of the study, three of them are family related, the father and two of his daughters (CNV of 2,8 Mb), and another patient from the population of study (CNV of 1,7 Mb). 16p13.11 microdeletion syndrome is a recently described syndrome characterized by developmental delay, microcephaly, epilepsy, short stature, facial dimorphism and behavioral problems. The prevalence is <1 / 1.000.000 and the inheritance is autosomal dominant (ORPHANET 261236). Moreover, in the DECIPHER database are reported four patients (267852, 514842, 400004 and 289196) with the 16p13.11 deletion syndrome with a similar size CNV (1,7 Mb) and the following phenotypes related to abnormalities of the nervous system, growth abnormality and abnormality of limbs: global developmental delay, seizure, Chiari type I malformation, lower cranial nerve dysfunction, intellectual disability, atypical behavior, failure to thrive, intrauterine growth retardation, severe short stature, abnormality of the ankle and postaxial polydactyl. The three relatives in our study who presented the 16p13.11 deletion syndrome (16p13.11p12.3) with a CNV of 2,8 Mb, have a father/daughters relationship and, therefore, it has been confirmed that the deletion in the chromosome 16 presented by both daughters is inherited from the father. One of the sisters also has a duplication in chromosome 14, specifically in the cytoband 14q11.2, but it is reported as not pathogenic because this region does not present any OMIM gene and there is not any relationship between the duplication and neurodevelopmental delay in the scientific literature. She manifests the following clinical features: psychomotor delay, cognitive difficulties and intellectual disability. The other sister expresses the following clinical manifestations: developmental delay, social impairments and learning disabilities. The father presents the following clinical features: psychological problems, anxiety and depression. The other patient from the population study has a deletion in the cytoband 16p13.11 with a CNV of 1,7 Mb and shows the following clinical manifestations: congenital hypotonia, neonatal respiratory failure, diaphragmatic paralysis (frenetic injury and diaphragmatic paresis), ostium secundum type atrial septal defect, feeding difficulties, percutaneous gastrostomy, left thigh incision, neonatal gastroesophageal reflux, cryptorquidia and campodactyly of the third finger on both hands. The CNV presents variable expressivity and incomplete penetrance, but as it is seen, all of the three relatives and the fourth patient of the study with the deletion on chromosome 16 have phenotype-genotype associations, therefore, it can be stated that the CNV is pathogenic.

- 17q12 microduplication syndrome: 17q12 microduplication syndrome is a rare chromosomal anomaly with variable phenotypic expression and reduced penetrance associated with developmental delay, mild to severe intellectual disability, speech delay, seizures, microcephaly, behavioral abnormalities, autism spectrum disorder, eye or vision defects (strabismus, astigmatism, amblyopia, cataract, coloboma and microphthalmia), non-specific dimorphic features, hypotonia, cardiac and renal anomalies, schizophrenia (OMIM 614526). The prevalence is <1 / 1.000.000 and the inheritance is unknown (ORPHANET 261272). The patient in our study who presented the duplication in the chromosome 17 manifests the following symptomatology related to the 17p12 microduplication syndrome described in the bibliography: syndromic phenotype and affected by autism spectrum disorder. Therefore, we can affirm that the CNV is pathogenic.

- 17q12 microdeletion syndrome: 17q12 microdeletion syndrome is a rare chromosomal anomaly syndrome resulting from the partial deletion of the long arm of chromosome 17 characterized by renal cystic disease, maturity onset diabetes of the young type 5 and neurodevelopmental disorders, such as cognitive impairment, developmental delay (particularly of speech), autistic traits and autism spectrum disorder. Müllerian aplasia in females, macrocephaly, mild facial dimorphism (high forehead, deep set eyes and chubby cheeks) and transient hyperkalemia have also been reported (OMIM 614527). The prevalence is <1 / 1.000.000 and the inheritance is unknown (ORPHANET 261265). The patient in our study who presented the deletion in the chromosome 17 manifests the following symptomatology related to the 17p12 microdeletion syndrome described in the bibliography: cognitive impairments, behavior problems and developmental delay. Therefore, we can affirm that the CNV is pathogenic.

- DiGeorge syndrome (DGS) or velocardiofacial syndrome (VCFS) is caused by a 1,5 to 3,0 Mb **heterozygous deletion in the *TBX1* gene on chromosome 22q11.21** and the inheritance is autosomal dominant. The clinical features are: short stature (20% of adults), obesity (35% of adults), micrognathia, low set ears, abnormal folded pinna, middle ear abnormalities, hearing deficits (28% of adults), posterior embryotoxon, tortuous retinal vasculature, hypertelorism, short palpebral fissures, eyelid hooding, amblyopia, strabismus (15% of adults), exotropia, esophoria, sclerocornea, accommodative exotropia, complicated strabismus, blunted nose, short philtrum, high arched palate, cleft palate, bifid uvula, cardiovascular malformations (26% of adults), tetralogy of Fallot, truncus arteriosus, interrupted aortic arch, right aortic arch, ventricular septal defect, patent ductus arteriosus, umbilical hernia, femoral hernia, cholelithiasis (19% of adults), inguinal hernia, unilateral renal agenesis, renal dysplasia,

hydronephrosis, scoliosis (47% of adults), severe acne (23% of adults), seborrhea (35% of adults), mild to moderate learning difficulties, delayed psychomotor development, late-onset speech development, tetany, seizures (40%), attention deficit and hyperactivity disorder, schizophrenia (22% of adults), bipolar disorder, hypernasal speech, parathyroid hypoplasia, parathyroid absence, thymic hypoplasia, thymic aplasia, accessory thyroid tissue, hypothyroidism (20% of adults), neonatal hypokalemia, hypokalemia (64% of adults), T-cell deficit, incidence is estimated to be between 1 in 2,000 and 1 in 7,000 live births and hernia occurs in 22% of adults (OMIM 601362). The DiGeorge syndrome has been diagnosed in three patients of the study. One of the patients is prenatal, therefore, no clinical manifestations are showed yet but the other two patients manifest the following clinical features related with the DGS: global development delay and mild cognitive impairment. As this is a well described genetic disorder, it can be stated that the CNV is pathogenic.

- 22q12.3q13.3 *de novo* terminal duplication: A duplication of 13,2 Mb in the 22q12.3q13.3 region has been reported as pathogenic in a prenatal patient. A study indicates that these alterations may be due to a *de novo* terminal duplication in the distal region of the long arm of chromosome 22 and are related to the following clinical characteristics: severe development delay, prenatal and postnatal growth retardation, cleft palate with or without cleft lip, micrognathia, microcephaly, hypertelorism, low set ears, congenital heart defects, renal and genital anomalies, skeletal abnormalities, hypotonia and the life expectancy appears to be highly reduce since almost half of all published patients have died before the age of 12 years (Brunetti Pierri N, et al. 2009). Moreover, in the DECIPHER database are reported two patients (398449 and 456159) with the *de novo* terminal 22q12.3 duplication with a similar size CNV and the following phenotypes related to abnormalities of the nervous system, growth abnormality and abnormality of the cardiovascular system: delayed speech, language development delay, expressive language delay, intellectual disability, intrauterine growth retardation, proportionate short stature, hypertrophic cardiomyopathy and pulmonic steatosis. The patient in our study that carries the duplication is prenatal and the aim for the array's petition is intrauterine growth retardation and suspected heart disease, in conclusion, the clinical manifestations described in the bibliography could explain the clinical context and we can affirm that the CNV is pathogenic.

- Duchenne-Becker muscular dystrophy (DMD and BMD) is caused by a **deletion in the *DMD* gene encoding dystrophin on chromosome Xp21.1** and the inheritance is X-linked recessive. Dystrophin-associated muscular dystrophies range from the severe Duchenne muscular dystrophy to the milder Becker muscular dystrophy. The clinical features are: Red-green eyed-color defect in many patients with deletion downstream of exon 30,

cardiomyopathy, congestive heart failure, pulmonary hypoventilation, respiratory failure, increased lordosis, scoliosis, flexion contractures, calf muscle pseudohypertrophy, weakness, mental retardation, hypotonia, waddling gait, hyporeflexia, positive gowers sign, high serum creatine kinase, abnormal electrocardiogram, absent dystrophin on muscle biopsy, usual onset before age 6 years and death by age 20, incidence of 1 in 3,500 boys, about 20% of female mutation carriers may show mild muscle weakness and about 8% of female mutation carriers develop dilated cardiomyopathy (OMIM 310200 and OMIM 300376). The Duchenne-Becker muscular dystrophy has been diagnosed in a patient of the study that is prenatal, therefore, no clinical manifestations are showed, but as this is a well described genetic disorder, it can be stated that the CNV is pathogenic.

- Klinefelter syndrome (KS) is **sex chromosome aneuploidy condition in which males have an extra X chromosome** (genotype XXY) compared to the 46,XY karyotype in typical males. The classic form of KS, which is present in the 80–90 % of the cases, is defined by a 47,XXY karyotype resulting from the aneuploidy of the sex chromosomes, whereas higher-grade aneuploidies (48,XXXY or 48,XXYY), structurally abnormal X chromosome (47,iXq,Y) or mosaicisms (47,XXY/46,XY) make up approximately in the remaining 10–20 % of cases. The clinical features are: tall stature, small testes, gynecomastia in late puberty, gynoid aspect of hips (broad hips), sparse body hair, signs of androgen deficiency and low serum testosterone coupled with elevated gonadotropins, and finally azoospermia, oligospermia with hyalinization and fibrosis of the seminiferous tubules. The above-mentioned signs of hypogonadism are also coupled with endocrine disorders such as osteoporosis, obesity and diabetes, musculoskeletal disorders, cardiovascular disorders, autoimmune disorders, cancer, neurocognitive disabilities, neuropsychological difficulties, language and speech problems and infertility (Bonomi M, et al. 2017). The Klinefelter Syndrome has been diagnosed in a patient of the study who manifest the following clinical features related with the KS: hypertonia in the lower extremities and stagnant head circumference. As this is a well described genetic disorder, it can be stated that the CNV is pathogenic.

- Triple X syndrome or X trisomy is a **sex chromosome aneuploidy condition in which females have an extra X chromosome** (genotype XXX), compared to the 46,XX karyotype in typical females. Although nonmosaic 47,XXX karyotypes are the most frequent, mosaicism occurs in approximately 10% of cases and can occur in many combinations such as 46,XX/47,XXX or 47,XXX/48,XXXX, or in combinations including Turner syndrome cell lines such as 45,X/47,XXX or 45,X/46,XX/ 47,XXX. The clinical features are: epicanthal folds, hypertelorism, upslanting palpebral fissures, clinodactyly, overlapping digits, pes planus, pectus excavatum, hypotonia, joint

hyperextensibility, tall stature, long legs, genitourinary abnormalities, unilateral kidney, renal dysplasia, ovarian malformations, congenital heart defects, atrial and ventricular septal defects, pulmonic stenosis, aortic coarctation, seizures, gastrointestinal problems, ovarian or uterine digenesis, premature ovarian failure, autoimmune thyroid disorder, developmental delays such as speech-language development and motor development related to hypotonia, developmental dyspraxia, cognitive deficits, learning disabilities, attention deficit and hyperactivity disorder (in 25-35% of cases), anxiety, depression/dysthymia and psychotic disorders (Tartaglia NR, et al. 2010). The triple X syndrome has been diagnosed in a patient of the study who manifest the following clinical features related with the X trisomy: moderate/severe autism spectrum disorder, sleep disorder and eating disorder. As this is a well described genetic disorder, it can be stated that the CNV is pathogenic.

- A deletion of 21,5 Mb in the region Xp22.33p22.12, a duplication of 36,7 Mb in the region Xp22.12p11.1 and a duplication of 7,1 Mb in the region 9p24.3p24.1 have been reported as pathogenic in a patient of the study. In the scientific literature it is reported that patients with deletions on the short arm of chromosome X have short stature and may have some somatic traits typical of Turner syndrome (TS) whereas gonadal function is generally preserved. In a study were it is described an analysis of a family with a deletion in the cytoband Xp22.33p22.12, the mother and both daughters had only a short stature, a skeletal survey showed normal findings except for mildly shortened 4th and 5th metacarpal bones and no features of TS were present (Cho SY, et al. 2012). In a recent study, an 11-year-old female who was recently diagnosed with TS, the chromosome single nucleotide polymorphism (SNP) array revealed microdeletion of Xp22.33p22.12. Both the patient and her mother have short stature and her mother, however, has a normal karyotype. This is one of few case reports of TS with microdeletion of Xp22.33p22.12 reported in the literature, with normal ovarian function and possible future transmission of the deletion to the next generations and this deletion on the short arm of chromosome X has been reported once previously in the literature. About 50% of affected cases are monosomic for X chromosome and tend to present with short stature/skeletal changes, a webbed neck, cardiovascular and renal abnormalities, gonadal digenesis and/or ovarian failure (D'Ambrosio F, et al. 2019). In the DECIPHER database are reported two patients (258916 and 258134) with the Xp22.12p11.1 duplication with a similar size CNV (36,7 Mb) and the following clinical manifestations: heterochromia iridis, congenital nystagmus, visual impairment, downslanted palpebral fissures, long eyelashes, short foot, short palm, plagiocephaly and intellectual disability. Recent studies suggest that duplication of the 9p24.3 chromosomal locus, which includes the *DOCK8* and *KANK1* genes, is associated with autism spectrum disorders, intellectual

disability, developmental delay, learning problems, language disorders, hyperactivity and epilepsy (Capkova Z, et al. 2021). The patient in our study expresses a Turner syndrome phenotype and an altered cytogenetic study 46,X,add(X)(p22.1). There are correlations between the patient's phenotype and the clinical manifestations informed in the bibliography: typical traits from Turner syndrome. Therefore, we can affirm that all the CNVs described are pathogenic.

### 7.2. ACCIDENTAL DISCOVERY

In the 48 positive samples from patients with genetic alterations, there is an accidental discovery:

- Charcot-Marie-Tooth Disease Type 1A (CMT1A) is caused by a **duplication in the *PMP22* gene encoding peripheral myelin protein 22 on chromosome 17p12** and the inheritance is autosomal dominant. The clinical features are: kyphoscoliosis may occur, claw hand deformities in severe cases, pes cavus, hammer toes, foot deformities, distal limb muscle weakness due to peripheral neuropathy, distal limb muscle atrophy due to peripheral neuropathy, 'steppage' gait, foot drop, cold-induced muscle cramps, distal sensory impairment, hyporeflexia, areflexia, decreased motor nerve conduction velocity (less than 38 m/s), hypertrophic nerve changes, 'onion bulb' formations seen on nerve biopsy, segmental demyelination/remyelination seen on nerve biopsy, decreased number of myelinated fibers, myelin outfoldings (in some patients), onset in first or second decade, usually begins in feet and legs (peroneal distribution), upper limb involvement usually occurs later, slowly progressive, insidious onset, variable severity, allelic disorders with overlapping phenotypes include Dejerine-Sottas Syndrome, hereditary neuropathy with liability to pressure palsies and Charcot-Marie-Tooth with deafness (OMIM 118220). The aim for the array's petition is behavior disorder, language delay and hyperactivity. The clinical manifestations described in the bibliography, CMT1A due to a duplication in 17p12, do not explain the clinical context of the patient, so this clinical case is reported as an accidental discovery. This is because the patient attended to the medical appointment due to behavior disorder, language delay and hyperactivity and the genetic results showed that the duplication in the chromosome 17 causes CMT1A, which clinical features manifest in an older age, in conclusion, we can affirm that the CNV is pathogenic but it is due to an accidental discovery that does not explain the clinical manifestations of the patient yet.

## 7.3. VOUS

Considering the 26 results obtained classified as of uncertain significance, there is one possibly benign polymorphism, 16 possibly pathogenic CNVs and 9 results that do not explain the clinical context of the patient.

### 7.3.1. VOUS LIKELY BENIGN

- A deletion of 580 Kb in the region 6p12.3 and a duplication of 80 Kb in the region Yq11.22 have been reported as a probably benign polymorphism in a patient. The deletion on chromosome 6 is reported as not pathogenic because this region does not present any OMIM gene and there is not any relationship between the duplication and neurodevelopmental delay in the scientific literature. The duplication on chromosome Y affects *TTTY5* gene, but it is reported as probably benign due to there is no clear evidence that relates the duplication to any known syndrome. The patient in the study manifests the following clinical features: intellectual disability, language delay and learning difficulties. No alterations in the CNVs have been detected, with the level of resolution used, that could explain the phenotype that motivated the consultation. In conclusion, it is reported as an uncertain significance that does not explain the clinical context of the patient because it is probably a benign polymorphism.

### 7.3.2. VOUS LIKELY PATHOGENIC

- 2p16.3 deletion syndrome: 2p16.3 deletion syndrome is caused by a deletion in the *NRXN1* gene on the short arm of the chromosome 2 which is associated with susceptibility to autism spectrum disorder and schizophrenia. The clinical features are: autism spectrum disorder, schizophrenia, developmental delay, intellectual disability, and dimorphic features. The phenotype shows incomplete penetrance and variable expression. In a study with 17 patients with exonic deletions showed a wide range of phenotypes, including delayed psychomotor development/intellectual disability (93%), infantile hypotonia (59%), autism spectrum disorders (56%), and seizures (53%). Attention deficit-hyperactivity disorder was also commonly observed. Congenital malformations and dimorphic features were not consistent. Neither the prevalence or the inheritance have been previously described, but the study shows that 3 deletions occurred *de novo* and 9 were inherited from a parent, also 8 of the 9 parents from whom a deletion was inherited had a history of learning disabilities and/or neuropsychiatric disease (OMIM 614332). In the DECIPHER database are reported two patients (437859 and 424284) with the deletion in the chromosome 2 with a similar size CNV (190 Kb) and the following phenotypes related to abnormalities of the nervous system: intellectual disability, atypical behavior and global developmental delay. The

patient manifests the following clinical symptomatology: development delay and autism spectrum disorder. Although the CNV found shows incomplete penetrance and variable expression, there are associations between the bibliography and the clinical manifestations from the patient in our study. In conclusion, it is a CNV reported as an uncertain significance that could explain the clinical context of the patient.

- 3q29 microdeletion syndrome: 3q29 microdeletion syndrome is caused by a recurrent deletion of the 3q subtelomeric region. The microdeletion is commonly 1.6 Mb in length and encompasses more than 20 genes. The clinical phenotype is extremely variable. The most common features include mild-to-moderate intellectual deficit and slightly dimorphic facial features: microcephaly, long and narrow face, short philtrum, large posteriorly rotated ears and high nasal bridge. Autism spectrum disorder and gait ataxia have been noted occasionally. Congenital malformations are not common: there are only rare reports of horseshoe kidney, hypospadias and congenital heart defects (patent ductus arteriosus) (OMIM 609425). The prevalence is unknown and the inheritance is autosomal dominant (ORPHANET 65286). In the DECIPHER database is reported a patient (274996) with the deletion in the chromosome 3 with a similar size CNV (621 Kb) and the following phenotypes related to abnormalities of the nervous system: attention deficit hyperactivity disorder and intellectual disability. The patient in our study manifests the following clinical symptomatology: behavior disorder, severe intellectual disability and familial paroxysmal ataxia. Based on the scientific literature research, we can affirm that the clinical manifestations described in the bibliography, mostly intellectual disability and ataxia, are similar to the patient's features. In conclusion, it is a CNV reported as an uncertain significance that could explain the clinical context of the patient.

- 4p15.32 *de novo* interstitial duplication: A duplication of 970 Kb and 946 Kb in the 4p15.23 region has been reported as likely pathogenic in two patients of this study. A recent paper indicates that these alterations may be due to a *de novo* interstitial duplication in the short arm of chromosome 4 and are related to the following clinical characteristics: macrocephaly, broad nasal bridge, hypertelorism, epicanthal folds, coloboma of the eye, intellectual disability, epilepsy, renal anomalies, hypotonia, growth retardation, hypoplastic genitalia/hypospadias, hypoplasia of the corpus callosum and diabetes mellitus (Zhang X, et al. 2023). Moreover, in the DECIPHER database are reported three patients (327263, 366362 and 500792) with the *de novo* interstitial 4p12.32 duplication with a similar size CNV and the following phenotypes related to abnormalities of the nervous system: intellectual disability, global development delay and autism spectrum disorder. The two patients in our study who presented the 4p15.32 *de novo* interstitial duplication are not family related but they present similarities in the

symptomatology and could explain the clinical context, even though there is not much bibliography that describes the clinical manifestations. The first patient presents attention deficit and hyperactivity disorder, memory concentration and orientation disorders, suspicion of mild cognitive delay and the second patient presents development delay and cognitive impairment. In conclusion, it is a CNV reported as an uncertain significance that could explain the clinical context of both patients.

- Trisomy 5p: Trisomy 5p is a chromosomal abnormality resulting from the duplication of a segment of variable size of the short arm of chromosome 5, which usually involves the distal band 5p15. The clinical presentation is variable but is always associated with severe intellectual deficit (ORPHANET 1742). In the DECIPHER database are registered three patients (326667, 339776 and 349786) with the duplication in the chromosome 5 with a similar size CNV (2 Mb). The first one presents an abnormality of the voice (cat cry) and the other two have phenotypes related to abnormalities of the nervous system such as autism spectrum disorder and intellectual disability. It is known that this region partially overlaps with the cri du chat syndrome (deletion on the short arm of chromosome 5, classically characterized by cat-like cry and associated with varying degrees of intellectual disability, developmental delay, microcephaly and facial dimorphism) but it is a duplication instead. Even though there is not much bibliography that describes this duplication in the chromosome 5, this CNV (5p15.1p14.3) could explain the clinical context of the patient: neurodevelopment delay and epilepsy.

- 8p23.1 duplication syndrome: 8p23.1 duplication syndrome is a rare chromosomal anomaly syndrome, resulting from the partial duplication of the short arm of chromosome 8, with a highly variable phenotype, principally characterized by mild to moderate developmental delay, intellectual disability, mild facial dimorphism (including prominent forehead, arched eyebrows, broad nasal bridge, upturned nares, cleft lip and/or palate) and congenital cardiac anomalies (atrioventricular septal defect). Other reported features include macrocephaly, behavioral abnormalities (attention deficit disorder), seizures, hypotonia and ocular and digital anomalies (poly/syndactyly). The prevalence is 1-9 / 100.000 and the inheritance is unknown (ORPHANET 251076). In the DECIPHER database are reported three patients (296542, 391626 and 391420) with the duplication in the chromosome 8 with a similar size CNV (525 Kb). The first one manifests intellectual disability and the other two have phenotypes related to abnormalities of the cardiovascular system (abnormal heart valve morphology, bicuspid aortic valve and aortic valve stenosis). The two patients in our study who presented the 8p23.1 duplication syndrome have a mother/fetus relationship and, therefore, it has been confirmed that the duplication in the chromosome 8 presented by the fetus is inherited from the mother, and as long as the region involved in the CNV is

incomplete penetrance and variable expressivity, could explain the clinical context but it is reported as an uncertain significance: the fetus presents polyhydramnios and the mother doesn't express any symptomatology.

- Mosaic trisomy 9: Mosaic trisomy 9 is a rare chromosomal anomaly syndrome, with a highly variable phenotype, principally characterized by intellectual disability, growth and developmental delay, facial dimorphism (microphthalmia, deep-set eyes, low-set, malformed ears, bulbous nose, high-arched palate, micrognathia) and congenital heart defects (ventricular septal defect), as well as urogenital (hypoplastic genitalia and cryptorchidism), skeletal (congenital joint dislocations or hyperflexion, scoliosis/kyphosis) and central nervous system anomalies (hydrocephalus, Dandy-Walker malformation). Pigmentary mosaic skin lesions along the lines of Blaschko are also frequently observed (ORPHANET 99776). The patient in our study presents a low-level mosaic trisomy 9 and by using the FISH technique it was confirmed that the mosaicism level is 5%. The patient manifests the following clinical symptomatology based on a peculiar phenotype: thymic hypoplasia, silver hair, short limbs, scaphocephaly, dolichocephaly, flat nasal root, small sunken eyes, low-set ears, prominent forehead, underdeveloped genitalia and empty scrotal sacs. Although the duplication of chromosome 9 is reported as an uncertain significance, the bibliography could explain the clinical context of the patient.

- 15q13.3 microduplication syndrome: 15q13.3 microduplication syndrome affects the *CHRNA7* gene, a strong candidate for the behavioral abnormalities, and it is associated with a wide spectrum of clinical presentations ranging from normal to different neuropsychiatric conditions, such as developmental delay, intellectual disability, epilepsy, hypotonia, autism spectrum disorders, attention deficit and hyperactivity disorder and schizophrenia. A strong candidate for the behavioral abnormalities (Budisteanu M, et al. 2021). In the DECIPHER database is reported a patient (269230) with the duplication in the chromosome 15 with a similar size CNV (2,47 Mb) and the following phenotypes related to abnormalities of the nervous system: dyslexia and intellectual disability. The patient manifests the following clinical symptomatology: global development delay, autism spectrum disorder with cognitive impairment, macrocephaly and intellectual disability. The patient also presents a duplication in the cytoband 22q11.23, and although the detected alteration involves the *SPECC1L* gene related with Opitz G/BBB Type II caused by a deletion, in the recent scientific literature there is not any evidence that relates a duplication with the clinical context. In conclusion, it is a CNV reported as an uncertain significance that could explain the clinical context of the patient.

- 16p12.2 microdeletion syndrome: 16p12.2 microdeletion syndrome is caused by a heterozygous deletion on the short arm of chromosome 16 characterized by an extremely variable clinical phenotype and with incomplete penetrance and variable expression. Common characteristics that have been described include developmental delay, delayed speech, intellectual disability that ranges from mild to profound, weak muscle tone (hypotonia), slow growth resulting in short stature, usually small head (macrocephaly) malformations of the heart, recurrent seizures (epilepsy), and psychiatric and behavioral problems. Findings commonly observed in children (probands) with this deletion include: developmental delay, cognitive impairment (ranging from mild to profound), growth impairment (including short stature), cardiac malformations, epilepsy, and psychiatric and/or behavioral issues. Other findings can include: hearing loss, dental abnormalities, renal and genital anomalies (the latter in males), cleft palate and cleft lip (Girirajan S, et al. 2015). In the DECIPHER database are reported two patients (262045 and 386899) with the deletion in the chromosome 16 with a similar size CNV (448 Kb) and the following phenotypes related to abnormalities of the nervous system: autism spectrum disorder, delayed speech and language development. The patient in our study manifests the following clinical symptomatology: autism spectrum disorder and epilepsy. The clinical features found in the scientific literature correlate with the clinical characteristics expressed by the patient in our study, but this CNV is incomplete penetrance and variable expression, some patients do not express any symptomatology and other patients manifest severe clinical features. In conclusion, it is a CNV reported as an uncertain significance that could explain the clinical context of the patient.

- 16q23.2 *de novo* deletion: A duplication of 419 Kb in the 16q23.2 region has been reported as likely pathogenic in a patient of this study. A paper shows that these alterations may be due to a *de novo* deletion in the long arm of chromosome 16 that involves two genes, *GAN* and *CMIP*, implicated in specific language impairment and autism spectrum disorder. In a developmentally delayed girl with an autism spectrum disorder, single nucleotide polymorphism (SNP) array analysis showed a *de novo* 280 Kb deletion on chromosome 16q23.2 involving two genes, *GAN* and *CMIP*. Inactivating mutations in *GAN* cause the autosomal recessive disorder giant axonal neuropathy, not present in our patient. Haploinsufficiency of *CMIP* was recently implicated in the etiology of specific language impairment and it modulates phonological short-term memory and hence plays an important role in language acquisition. Overlaps of specific language impairment and autism spectrum disorder have been debated in the literature regarding the phenotypical language profile as well as etiology (Van der Aa N, et al. 2012). In the DECIPHER database is reported a patient (282031) with the deletion in the chromosome 16 with a similar size CNV and the following phenotypes

related to abnormalities of the nervous system: attention deficit and hyperactivity disorder, autism spectrum disorder, delayed fine motor development, delayed speech and language development, global developmental delay and intellectual disability. The patient in our study manifests the following clinical symptomatology: cognitive impairment, language delay and epilepsy. Even though the main clinical manifestation described in the scientific literature is autism spectrum disorder and the patient in our study does not manifest it, the other clinical affectations manifested by the patient are justified by the bibliography. In conclusion, it is a CNV reported as an uncertain significance that could explain the clinical context of the patient.

- Smith-Magenis syndrome (SMS) is caused by a **deletion in the *RAI1* gene on chromosome 17p11.2** and the inheritance is autosomal dominant. A duplication of 300 Kb in the 17p11.2 region involving *AKAP10* gene and *SPECC1* gene has been reported as an uncertain significance but likely pathogenic in a patient of this study. 17p11.2 deletion syndrome, also known as Smith-Magenis syndrome, is caused in most cases (90%) by a 3,7 Mb interstitial deletion in chromosome 17p11.2 and common characteristics in the nervous system that have been described include: speech delay, mental retardation (IQ 20-78), sleep disturbance, structural brain abnormalities, peripheral neuropathy, decreased pain sensitivity, normal nerve conduction velocities, decrease/absent deep tendon reflexes, hyperactivity, polyembolokoilamania (insertion of foreign bodies into body orifices), behavioral problems, self-destructive behavior, onychotillomania (pulling out nails), wrist-biting and head-banging (OMIM 182290). However, as mentioned before, the patient in our study shows a deletion in the short arm of the chromosome 17 of 300 Kb and the scientific literature affirms that most cases show a deletion of 3,7 Mb. It is important to note that the interpretation of this clinical case must be careful. Moreover, in the DECIPHER database is reported a patient (497319) with the 17p11.2 duplication with a CNV of 30 Kb and the following phenotypes related to abnormalities of the nervous system: delayed speech and language development and global developmental delay. The patient in our study manifests the following clinical symptomatology: autism spectrum disorder, speech delay and café-au-lait spots. Based on the scientific literature research, we can affirm that the clinical manifestations described in the bibliography, mostly delayed speech, are similar to the patient's features. In conclusion, even though there is not much bibliography that describes this deletion in the chromosome 17 with that specific size (300 Kb), this CNV (17p11.2) reported as an uncertain significance could explain the clinical context of the patient.

- 22q11.2 duplication syndrome: 22q11.2 duplication syndrome is a rare chromosomal anomaly characterized by an extremely variable clinical

phenotype and may include heart defects, urogenital abnormalities, velopharyngeal insufficiency with or without cleft palate, and ranging from multiple defects to mild learning difficulties with some individuals being essentially normal (OMIM 608363). The prevalence is unknown and the inheritance is autosomal dominant (ORPHANET 1727). In the DECIPHER database are reported three patients (326483, 331028 and 390132) with the duplication in the chromosome 22 with a similar size CNV (2,76 Mb) and the following phenotypes related to abnormalities of the nervous system: specific learning disability, global development delay, dysplastic corpus callosum and severe intellectual disability. It is known that this region partially overlaps with the DiGeorge syndrome (deletion on the long arm of chromosome 22 related with neurodevelopment disorders and a defined phenotype with dimorphic features) but it is a duplication instead. The patient manifests the following clinical symptomatology: language delay, intellectual disability and hyperkinesia. The patient also presents a duplication in the pseudoautosomal region in chromosome X or Y, specifically in the cytoband Xp22.33 or Yp11.32, but it is reported of being uncertain significance due to there is no clear evidence that relates the duplication to any known syndrome and there is not any similar duplication described in the healthy population or in the polymorphism databases. This chromosomal region involving the duplication in the chromosome 22 it is known for being incomplete penetrance and variable expressivity, where there are some patients completely asymptomatic and other patients with severe clinical manifestations, in conclusion, it is a CNV reported as an uncertain significance that could explain the clinical context of the patient.

- Xp22.31p22.2 duplication syndrome: A duplication of 240 Kb, 1,5 Mb and 1,6 Mb in the Xp22.31 region has been reported as likely pathogenic in three patients of this study. Xp22.31p22.2 duplication syndrome is a rare syndromic intellectual disability characterized by developmental delay and intellectual disability, learning and behavioral problems, short stature, thin and sparse hair, mild dimorphic features, tapering fingers and later onset of scoliosis, obesity and cardiovascular problems (cardiomegaly and cardiomyopathy). Females have normal intelligence. The Xp22.31 duplication presents variable expressivity and incomplete penetrance. The prevalence is <1 / 1.000.000 and the inheritance is X-linked recessive (ORPHANET 284180). In the DECIPHER database are reported two patients (1941 and 292839) with the duplication in the chromosome X with a similar size CNV (204 Kb) and the following phenotypes related to abnormalities of the nervous system: intellectual disability and cognitive impairment. Both of these patients present the duplication in the chromosome X that involves *STS* gene, as well as the three patients reported in our study, and it is known that genetic affections in this gene cause ichthyosis. The first patient with a CNV of 204 Kb manifests the following clinical symptomatology: learning disabilities and global

development delay. The second patient with a CNV of 1,5 Mb shows the following clinical manifestations: autism spectrum disorder. In both patients, the CNV was informed as an uncertain significance that could explain the clinical context of the patient. In contrast, the third patient with a CNV of 1,6 Mb presents the following clinical features: psychomotor delay, hypotonia and atrial septal defect. The phenotype expressed by the third patient had an association with the duplication in chromosome X identified with the aCGH technique, moreover, a complete sequencing study NGS (Next Generation Sequencing) was carried out and the results showed a variant in the *KDM6A* gene classified as pathogenic in the Kabuki syndrome panel. Kabuki syndrome is a congenital mental retardation syndrome with additional features, including short stature, low weight, congenital heart disease, atrial septal defect, atrioventricular septal defect (rare), pulmonary valve stenosis (rare), hypoplastic right ventricle (rare), aortic coarctation, developmental delay (mild to severe), impaired intellectual development (mild to severe), speech delay, motor delay, hypotonia, seizures, ventriculomegaly, delayed myelination (uncommon) and behavioral difficulties (OMIM 300867).

#### 7.3.3. VOUS THAT DO NOT EXPLAIN THE CLINICAL CONTEXT

- The results of the aCGH technique on a prenatal patient, with intracardiac hyperechogenic focus and single umbilical artery as symptomatology, revealed a duplication of 2 Mb in 1q21.1q21.2 and a deletion of 1,3 Mb in 5q21.2q21.3. A patient affected by autism spectrum disorder, global developmental delay and hyperkinesia presents a deletion of 90 Kb in the cytoband 1q25.1. A patient with a global developmental delay probably caused by a genetic disorder presents a duplication of 1,8 Mb in the chromosomal region 2q14.3. A patient with a global developmental delay probably caused by a genetic disorder presents a deletion of 737 Kb in the chromosomal region 3p12.3. A patient with a suspect of global developmental delay probably caused by a genetic disorder presents a duplication of 946 Kb in the chromosomal region 9p23. The results of the aCGH technique on a patient with an atrial septal defect, peculiar phenotype and dimorphic features suggestive from Rubinstein Taybi syndrome as symptomatology revealed a duplication of 922 Kb in 14q31.3. A patient affected by autism spectrum disorder presents a duplication of 160 Kb in the cytoband 15q13.1. A patient with a global developmental delay probably caused by a genetic disorder presents a deletion of 643 Kb in the chromosomal region 16q22.1. All of the CNVs previously mentioned, neither present any OMIM gene or there is not any relationship between the gain or the loss of genetic material and neurodevelopmental delay in the scientific literature. All those alterations are not described as a direct cause of any type of clinic, so the CNVs are defined as of uncertain significance that do not explain the clinical context. There is another patient with a duplication of 270 Kb in the cytoband Xq27.1 affected by autism spectrum disorder probably caused by a genetic disorder but the

CNV it is also classified as uncertain significance that do not explain the clinical context. A complete sequencing study NGS (Next Generation Sequencing) was carried out and the results showed a *de novo* variant in the *KMT2A* gene classified as pathogenic in the Wiedemann – Steiner syndrome panel. Wiedemann – Steiner syndrome is a rare genetic multiple congenital anomalies/dimorphic syndrome characterized by delayed psychomotor development, mental retardation, seizures, wide-based gait, speech delay, aggressive behavior and autistic features (OMIM 605130).

### 7.4. COST-EFFECTIVENESS ANALYSIS

As it was mentioned previously, the aCGH 60K platform offers a minimum resolution of 100 Kb in syndromic regions and 50 Kb in critical genes with an average resolution of 300 - 350kb in the rest of the genome. In contrast, the aCGH 180K platform offers a minimum resolution of 75 Kb in syndromic regions and 40 Kb in critical genes with an average resolution of 100kb in the rest of the genome. In addition, the aCGH 180K specifically designed for the study of ASD has a powerful increase in the detection capacity with a resolution of 15 Kb in 115 genes associated with ASD. Considering the 28 positive tests out of the 215 done with the aCGH 180K platform, only 4 resulted in a CNV less than 300 Kb:

- The **deletion of 90 Kb in the cytoband 1q25.1 detected with the aCGH 180K platform** affects the *TNR* gene, which it is associated to neurodevelopmental disorders but the inheritance is autosomal recessive. The patient expresses autism spectrum disorder, global developmental delay and hyperkinesia. These clinical manifestations could be due to a genetic cause only if both genes on both chromosomes are affected, because the inheritance is autosomal recessive. Therefore, a point mutation study should have been done in order to determine if it is affected and the patient expresses the neurodevelopment disorders due to the deletion of the *TNR* gene. It is classified as a VOUS that does not explain the clinical context and as the *TNR* gene is considered as a critical gene, it could have been detected with the aCGH 60K platform because it offers a minimum resolution of 50 Kb in critical genes.

- The **duplication of 160 Kb in the cytoband 15q13.1 detected with the aCGH 180K platform** does not present any OMIM gene and there is not any relationship between the duplication and neurodevelopmental delay in the scientific literature. Therefore, it could not have been detected with the aCGH 60K platform but as it is classified as a VOUS that does not explain the clinical context of the patient and does not have any OMIM gene, it is not related with the ASD manifested by the patient and it does not present a clinical significance.

- The **duplication of 204 Kb in the cytoband Xp22.31 detected with the aCGH 180K platform** affects the *STS* gene, *PUDP* gene and *MIR4767* gene which are associated to ichthyosis. Also, intellectual disability and autism spectrum disorder have been described. The patient manifests the following clinical symptomatology: learning disabilities and global development delay. This region affected is involved in the contiguous gene deletion ichthyosis (OMIM 308100), therefore, it could have been detected with the aCGH 60K platform because it offers a minimum resolution of 100 Kb in syndromic regions.

- The **duplication of 80 Kb in the cytoband Yq11.22 detected with the aCGH 180K platform** affects the *TTTY5* gene but it is reported as probably benign polymorphism due to there is no clear evidence that relates the duplication to any known syndrome. The patient expresses intellectual disability, language delay and learning difficulties. Therefore, it could not have been detected with the aCGH 60K platform but as it is classified as a probably benign polymorphism, it is not related with the intellectual disability, language delay and learning difficulties manifested by the patient and it does not present a clinical significance.

*Table 5. Analysis of whether the CNVs detected with the aCGH 180K platform could have been detected with the aCGH 60K platform*

| **CNV aCGH 180 K** | **It could have been detected with aCGH 60K (CNV < 300 Kb)?** | **It has clinical significance?** |
|---|---|---|
| 1q21.1q21.2 x3 (2,9 Mb) | Yes | Yes (Pathological) |
| 2p25.3 x1 (2,23 Mb) | Yes | Yes (Pathological) |
| 7q11.23 x3 (1,9 Mb) | Yes | Yes (Pathological) |
| 16p11.2 x1 (1,6 Mb) | Yes | Yes (Pathological) |
| 16p13.11 x1 (2,8 Mb (3)) | Yes | Yes (Pathological) |
| 17q12 x1 (1,4 Mb) | Yes | Yes (Pathological) |
| 22q11.21 x1 (2,8 Mb (2)) | Yes | Yes (Pathological) |
| 47,XXX | Yes | Yes (Pathological) |
| Xp22.33p22.12 x1 (21,5 Mb)<br>Xp22.12p11.1 x3 (36,7 Mb)<br>9p24.3p24.1 x3 (7,1 Mb) | Yes | Yes (Pathological) |
| 17p12 x3 (1,3 Mb) | Yes | Yes (Accidental discovery) |
| 6p12.3 x1 (580 Kb)<br>**Yq11.22 x3 (80 Kb)** | Yes (6p12.3 x1 580 Kb)<br>and **no (Yq11.22 x3 80 Kb) (CNV < 300 Kb)** | **No, it is a VOUS classified as a probably benign polymorphism because there is no clear evidence that relates the *TTTY5* gene to any known syndrome** |
| 4p15.32 x3<br>(970 Kb and 946 Kb) | Yes | Yes (VOUS likely pathogenic) |
| 15q13.2q13.3 x3 (2,47Mb)<br>22q11.23 x3 (1,32 Mb) | Yes | Yes (VOUS likely pathogenic) |
| 17p11.2 x1 (300 Kb) | Yes | Yes (VOUS likely pathogenic) |
| 22q11.21 x3<br>(2,73 Mb)<br>Xp22.33/Yp11.32 x2<br>(340 Kb) | Yes | Yes (VOUS likely pathogenic) |

| CNV aCGH 180 K | It could have been detected with aCGH 60K (CNV < 300 Kb)? | It has clinical significance? |
|---|---|---|
| **Xp22.31 x3 (1,6 Mb and 204 Kb)** | Yes (Xp22.31 x3 1,6 Mb) and **yes (Xp22.31 x3 204 Kb) because the region affects the *STS* gene which is associated to ichthyosis, therefore, it could have been detected with the aCGH 60K platform because it offers a minimum resolution of 100 Kb in syndromic regions** | **Yes (VOUS likely pathogenic)** |
| **1q25.1 x1 (90 Kb)** | **Yes, because the region affects the *TNR* gene which is associated to NDD, and even though the inheritance is autosomal recessive, it could have been detected with the aCGH 60K platform because it offers a minimum resolution of 50 Kb in critical genes** | **No, it is a VOUS that does not explain the clinical context, and even though the *TNR* gene is associated to NDD, the inheritance is autosomal recessive, so does not present a clinical significance unless a point mutation study is done** |
| 2q14.3 x3 (1,8 Mb) | Yes | No, it is a VOUS that does not explain the clinical context because neither present any OMIM gene or there is not any relationship between the duplication and NDD in the scientific literature |
| 3p12.3 x1 (737 Kb) | Yes | No, it is a VOUS that does not explain the clinical context because neither present any OMIM gene or there is not any relationship between the deletion and NDD in the scientific literature |
| 9p23 x3 (946 Kb) | Yes | No, it is a VOUS that does not explain the clinical context because neither present any OMIM gene or there is not any relationship between the duplication and NDD in the scientific literature |
| 14q31.3 x3 (922 Kb) | Yes | No, it is a VOUS that does not explain the clinical context because neither present any OMIM gene or there is not any relationship between the duplication and NDD in the scientific literature |
| **15q13.1 x3 (160 Kb)** | **No** (CNV < 300 Kb) | **No, it is a VOUS that does not explain the clinical context because neither present any OMIM gene or there is not any relationship between the duplication and NDD in the scientific literature** |
| 16q22.1 x1 (643 Kb) | Yes | No, it is a VOUS that does not explain the clinical context because neither present any OMIM gene or there is not any relationship between the deletion and NDD in the scientific literature |

As a summary, in our study all the 215 tests for genetic impairments done with the array CGH 180K platform (negative and positive), except of 2 (Yq11.22 x3 80 Kb and 15q13.1 x3 160 Kb), could have been performed with the array CGH 60K platform, but these 2 CNVs that could not have been detected have not any clinical significance, whether it is copy number variant probably benign polymorphism because there is no clear evidence that relates the affected gene to any known syndrome (Yq11.22 x3 80) or it is a variant of uncertain significance that does not explain the clinical context because neither present any OMIM gene or there is not any relationship between the gain or the loss of genetic material and neurodevelopmental delay in the scientific literature (15q13.1 x3 160 Kb). These results correlate perfectly with the theoretical framework established by scientific literature, which indicate that array CGH 60K platform provide the highest cost-benefit performance.

In a recent study from the Array Unit of the Section on Clinical and Molecular Genetics of the Hospital Universitari Vall d'Hebron of Barcelona, a comparative genomic hybridization analysis was carried out in 1000 patients affected from NDD, ASD and/or ID analyzing the cost-benefit of the technique. The results from Castells Sarret N, et al. (2018), have determined that the comparison between both aCGH platforms (4×180K and 8×60K) clearly showed greater specificity of the 8×60K platform in the detection of pathogenic CNVs. For example, in a particular case a pathogenic deletion of 0,043 Mb was detected by only 4 probes with the 4×180K platform and with 11 probes using the 8×60K platform, and in another case, a pathogenic duplication affecting the entire *RAF1* gene was detected with 10 probes with the 4×180K platform and with 18 probes with the 8×60K platform. Therefore, the comparison of the main commercial platforms carried out in this multicenter study concluded that aCGH 8x60K platform is the one that provides the highest cost-benefit performance.

In another study from the National Genetics Reference Laboratory (Wessex), a comparison for cytogenetics array platforms for use in identifying copy number aberrations was carried on. The results indicate that 8x60K array platform provided significant advances in both processing and quality whilst achieving significant consumable cost savings. In addition to the optional resolution required for diagnostic aCGH, the consumable cost per sample is a critical factor when reaching a decision as to which platform to use. From this it can be seen that the 4x180K array is the most expensive while the 8x60K array is significantly cheaper. The price comparisons are based on the published retail prices and include costs of all consumables involved in aCGH. The migration from the 4x180k array platform to the 8x60k array platform improved breakpoint resolution of ~ 3 times with an overall consumable cost reduction of 46%. In conclusion, 8x60K array platform presents the maximum balance between detection rates and costs of the technique. Consequently, it is recommended to limit the study of aCGH based on the 8x60K design or similar techniques.

## 8. CONCLUSIONS

In conclusion, we can affirm that an implantation of the array CGH 60K platform instead of the array CGH 180K platform in the diagnosis of NDD could suppose a benefit in the cost-effectiveness of the technique. This statement is supported by the recent scientific literature, which concludes that aCGH 8x60K platform is the one that provides the highest cost-benefit performance and to migrate from the aCGH 180k platform to the aCGH 60k platform we can achieve an improved breakpoint resolution of ~ 3 times, with an overall consumable cost reduction of 46% (Castells Sarret N, et al. 2018; Huang S, et al. 2010).

In addition, by actualizing and implementing relevant information in the genetic databases can help in the interpretation of the results in order to facilitate the genetic diagnosis, improving the quality of life of patients with neurodevelopmental diseases.

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