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CLINICAL RELEVANCE OF COPY NUMBER VARIATIONS DETECTED BY ARRAY-CGH IN SIX PATIENTS WITH UNEXPLAINED NON-SYNDROMIC INTELLECTUAL DISABILITY

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CLINICAL RELEVANCE OF COPY NUMBER VARIATIONS DETECTED BY ARRAY-CGH IN SIX PATIENTS WITH UNEXPLAINED NON-SYNDROMIC INTELLECTUAL DISABILITY

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Keywords: Intellectual disability, array-CGH, copy number variation, genotype, phenotype, non-syndromic

ABSTRACT

Intellectual disability (ID) represents a health problem of great relevance for the public health services and for the families and is one of the most common neurodevelopmental disorders, affecting 1 to 3% of children. Epidemiological studies show that genetic mutations contribute in about 15% to the etiology in milder forms. When ID is present with other symptoms or physical features, the identification of the causative effect is not so difficult to educe. On the contrary, ID can be present as a sole clinical feature or with additionally subtle symptoms, sometimes difficult to diagnose. In about half the cases of ID, despite a thorough medical history and laboratory investigation widened, the etiology remains unknown. In recent years, submicroscopic structural variations in copy number, known as copy number variations (CNVs) of a specific chromosomal region, have been implicated in the etiology of ID. Novel high resolution, whole genome technologies, such as array-based comparative genomic hybridization (array-CGH), improve the detection rate of submicroscopic chromosomal aberrations, allowing the re-investigation of cases where conventional cytogenetics failed to detect any mutation. However, the application of this contemporary molecular technology has also resulted in the discovery of widespread CNVs in human genome and understanding the clinical significance of these alterations on phenotype variation has been a real challenge, especially in non-syndromic ID (NS-ID). Although it has already contributed to the etiological classification of a large portion of ID patients, sometimes it can be difficult to pinpoint for certain that the alterations found are the cause of the phenotype, especially in NS-ID cases. With the rapid discovery of the genetic causes underlying

NS-ID and the pathways and processes involved in neurodevelopment and cognition, this will inevitably feedback into clinical diagnostics of these forms of ID. The aim of this study is to use array-CGH for the screening of patients with "idiopathic" ID, in order to detect submicroscopic copy number changes and correlate them with the phenotype.

In this study, a sample of 42 children monitored at the Neurodevelopmental and Autistic Unit of the *Carmona da Mota* Pediatric Hospital of Coimbra, with the diagnosis of non-syndromic ID (NS-ID) or with minor dysmorphisms based on clinical observation and implementation of gold standard scales for ID diagnosis, are being analyzed by array-CGH (17 Mb resolution level) in Life and Health Sciences Research Institute (ICVS) from Minho´s University. According to available laboratorial results, we selected six patients from the sample of 42 to analyze whether the high resolution, genomewide, microarray-based, copy number screening would allow the identification of small rearrangements not detected by other techniques which could be the explanation for their NS-ID or learning disorders.

In these six patients, we found two likely pathogenic chromosomal alterations, the duplications in Xp11.22 and 2q11.2 chromosomes. Based on current evidence, other potential pathogenic alterations were found and in many others it was not possible to assess their clinical significance.

During the interpretation of the clinical relevance of CNVs, many generalizations are made, as regards to the type of the event, size, gene content and inheritance. Sometimes this can lead to the exclusion of CNVs that could have a real impact on the phenotype. Current evidence shows that small duplications can have a role in the phenotype and even alterations that do not encompass any genes may affect the genetic expression. Moreover, environmental factors may also have an impact on the way genotype is expressed. This study underlines the importance of a new integrated and multidimensional model for the interpretation of alterations found by array-CGH, specially in cases of NS-ID.

INTRODUCTION

Early onset intellectual impairment is a problem of great complexity that has attracted particular attention among scientists in the last decade after a stationary period, when intellectual deficiency (ID) was perceived as a social or educational issue instead of a neurological condition [1-2]. It represents a health problem of great relevance not only because it is a common condition but also because of the impact it has on children´s lives, and its consequences, no less important, on family dynamic and structure as well as a economic and social impact on society. However, there are still many unsolved issues related to ID that need to be answered, namely concerning etiology. Indeed, the gap between the diagnosis of ID early in childhood and the identification of its cause has attracted the attention of the scientific community in the search for answers and has raised high expectations in pediatricians and psychologists who work on daily basis with these children and their families. In the last years, the increasing interest around ID along with the development of molecular cytogenetics has revolutionized the clinical management of these patients as well as our knowledge about the genetic causes and mechanisms underlying ID and their implications for genetic counseling.

Intellectual disability, formerly known as mental retardation, is clinically characterized by significant limitations in cognitive functioning and adaptive behavior, as expressed in conceptual, social and practical adaptive skills, with onset before the age of 18 years old [3]. About 1 to 3% of the total population are reported to be functioning two standard deviations (SDs -15) below the mean (100) of global intellectual quotient (GIQ) of general population, i.e., below 70 [4-5]. Mild ID (IQ between 50-69) is the most frequent (up to 80-85% of all ID) [6]. In addition to categorization by severity/IQ level, ID can also be classified as syndromic ID (S-ID) or non-syndromic (or non-specific) ID (NS-ID) forms. In S-ID patients have one or multiple clinical features or co-morbidities in addition to ID, while patients with NS-ID have a sole clinical feature: cognitive impairment [7-8]. However, characterization of NS-ID has some challenges. Sometimes it is difficult to rule out the presence of more neurological anomalies and psychiatric anomalies in these patients and some symptoms or signs are so subtle that they might be missed, specially in the absence of a known genetic defect previously associated with these features.

Epidemiological studies show that ID affects approximately 30% more males than females [4, 8-10], a sex bias that has been explained by X-linked genes mutations [7]. However, this ratio difference decreases in lower IQ subpopulations [11]. Despite the transversal occurrence in every social class and culture, the prevalence of ID tends to be higher in poor countries, a fact that has been attributed to non-genetic causes such as malnutrition, cultural deprivation, poor health care and parental consanguinity [1], which is supported by the finding that inbreeding is associated with higher risk factor for cognitive impairment [12]. Actually, many factors can be involved in the pathogenesis of ID, mainly in mild forms, by affecting the normal development and functioning of the central nervous system at different stages of life - prenatally, perinatally or postnatally [6]. Unfortunately, despite the advance in basic neurosciences, ID etiology remains unknown in approximately half of the cases[13-15]. While environmental features can explain some cases of ID, a genetic cause must be considered in the majority of the patients with severe ID, accounting for 25 to 50% of this cases [7], and in around 15% of the patients with mild forms[16-17]. X-linked Intellectual Disability (XLID) is an important subgroup of monogenic ID, fragile X syndrome (FXS) being the most prevalent hereditary condition by far (~20% of all cases of XLID) [18]. Another origin that has to be considered is subtelomeric rearrangements, which are estimated to be responsible for 5 to 7% of all cases of ID [19-21]. Additionally, in recent years, copy number variations (CNVs) are being associated in a large number of studies in ID populations [22-23] as well as in other neurological disorders [24]. By definition, a CNV is a segment of DNA that is 1 kilo base pairs (Kb) or larger and is present at a variable copy number (gain - duplication or loss – deletion) in comparison with a reference genome [25]. Nevertheless, CNVs are also a predominant form of genetic diversity among humans, representing frequently benign polymorphism variants [25].

The genetic heterogeneity that underlies cognitive impairment is so vast that many times searching for a genetic etiology can become a real challenge. As far as is known, genetic imbalances are the most frequent causes behind ID [26] and cytogenetic analysis has been playing an important role in the work-up of these patients. Some of the genomic imbalances are large-scale chromosomal abnormalities that can be detected by conventional cytogenetic techniques. G-banding karyotyping can sometimes detect

genomin imbalances as small as 3 million base pairs (Mb), but it can often miss genetic imbalances in the 5 to 10 Mb range, depending on the genomic region and/or conditions of the array [27]. It has a detection rate of 3%, excluding Down syndrome and other clinically recognizable chromosomal syndromes [27]. In spite of its important role as a medical diagnostic tool, traditional cytogenetic methods cannot reliably detect these small genomic rearrangements [28], also known as cryptic chromosomal imbalances, which have become a significant etiological research field and some of the new causes for the previous "idiopathic ID". Meanwhile, the introduction of new molecular techniques, as fluorescent in situ hybridization (FISH) and multiple ligation-dependent probe analysis (MLPA) circumvented some of the limitations of traditional cytogenetics and have been used as adjuncts to routine cytogenetic testing in order to detect small specific subtelomeric changes or chromosomal abnormalities suspected based on clinical observation [23, 29-31].

Array-based comparative genomic hybridization (array-CGH) was developed more than a decade ago and represents an advance in molecular cytogenetic study which has improved the diagnostic yield in about 10% [17] . As array-CGH have significantly improved the detection rates of individuals with previous normal results, this new technique is being now recommended as a first-tier genetic test for patients with unexplained ID, autism or multiple congenital disorders [27, 32]. On the other hand, the high level of chromosome resolution (less than 1Mb) and the capacity to screen the entire genome has resulted in the identification of a wide number of copy number variations (CNVs) in both healthy individuals and in subjects with the most varied neurodevelopmental pathological conditions. Consequently, the presence of identical CNVs with different clinical symptomatology has complicated the interpretation of the results and the regular application of this method in clinical practice. Distinguishing benign CNVs from pathogenic ones is the most serious challenge to the routine clinical use of array-CGH [33]. This upcoming issue claims for a close combined results interpretation between laboratory researchers and clinicians from the outpatient clinic.

The guidelines currently adopted in Portugal for genetic testing of these patients suggest the search of genomic imbalances or rearrangements by regular G-banded karyotyping in first place, followed, whenever the result is normal, by analysis of subtelomeric rearrangements, using specific FISH probes according to clinical presentation, and by

the testing of the most common single gene disorders (for instance, Fragile X syndrome).

In this study, genome-wide array-CGH is being used to screen 42 children with "idiopathic" ID for chromosomal imbalances that conventional methods and other techniques failed to reveal. According to the results available and their meaning we first report six representative cases selected from the larger sample.

The aim of the study is to detect submicroscopic copy number changes and correlate them with the phenotype and, when possible, consider them as the putative cause of the child´s phenotype.

PATIENTS AND METHODS

PATIENTS

The six cases presented in this paper are part of a sample of 42 children diagnosed with ID, mostly non-syndromic or with minor dysmorphisms, or borderline ID of unexplained etiology. The patients were studied and are followed up by a multidisciplinary team from the Neurodevelopmental and Autism Unit of the *Carmona da Mota* Pediatric Hospital of Coimbra (HP). The team includes pediatricians with differentiation in neurodevelopment, psychologists and social assistant. The patients from this hospital were selected by this team. The study is pilot project research between HP and Life and Health Sciences Research Institute (ICVS) of the University of Minho (UM). All the children included needed to fulfill the criteria described in the Table1.

Table 1. Eligibility criteria for the studied patients. All the patients included in the study needed to fulfill the criteria described below.

Eligibility criteria for the studied patients
Inclusion criteria
1. Documented developmental delay/ ID on basis of an individually administered test for IQ or DQ test equal/below 70 for individuals with ages three or higher or borderline IQ (>70<85); 2. Dysfunction/impairment in more than two areas of: communication, self-care, home living, social/interpersonal skills, use of community resources, self direction, functional academic skills, work, leisure and safety. 3. Documented developmental delay on basis of clinical evaluation by a paediatrician for individuals with ages between one and three years of age. Individuals included in this group will be individually administered DQ test when they reach age of three. 4. Unknown etiology/unexplained developmental delay/ID. 5. Onset of the developmental delay/ID during childhood. 6. Previous normal investigation including: baseline general investigation including biochemistry workup (renal, liver, thyroid function tests) and full blood count; documented high-resolution karyotype (>650 bands); fragile X testing when clinically indicated (FRAXA and FRAXE); metabolic screen when clinical findings or laboratory abnormalities suggesting a metabolic disorder; fluorescence in situ hybridization (FISH) studies when specific syndrome suspected (e.g. VeloCadioFacial, Williams-Beuren, Smith-Magenis...); pregnancy TORCH serologies if available or Guthrie's 7. DNA of the proband and both parents available.
Exclusion criteria
1. Not meeting any of the inclusion criteria; 2. Presence of a recognizable pattern of anomalies (known syndrome or association); 3. Establishment of the etiology, or specific diagnosis during the ongoing study.

IQ – Intelligence quotient / DQ – Developmental quotient / TORCH -Toxoplasmosis, Other infections, Rubella, Cytomegalovirus, Herpes simplex virus

In order to standardize and to facilitate the access to the information between clinical and laboratorial research professionals, all data was registered in detail into a database where each child has a code that ensures anonymity and allows the collaborators to

share and discuss information (http://www.neurodevgen.org). A DNA bank from blood samples for patients and families was also established at ICVS-UM.

A consented form was assigned by the parents and the study was conducted according to international ethical standard (Appendix 1).

CLINICAL HISTORY

In the evaluation of the children and families, a complete and detailed clinical history was collected as the first and the most important step in the diagnostic process. The evaluation was carried out by an expert multidisciplinary neurodevelopmental team. In a brief way, the information registered in the database includes: demographics data; personal history (pre and neonatal history, diseases, hospital admissions, medications); neurodevelopment history (age of developmental milestones); family and social history (father and mother´s age, ethnicity, literacy, history of psychopathology, development problems and other diseases, history of brothers and/or sisters with psychopathology and/or development problems); physical examination (weight, height, head circumference, dysmorphic signs, skin abnormalities, neurologic examination and sensorial evaluation).

DIAGNOSTIC TOOLS

INTELLECTUAL AND FUNCTIONAL ASSESSMENT

Standardized tests were routinely used to evaluate neurodevelopmental disorder and to diagnose ID level, such as *Griffiths Developmental Scale* [34] for children between two and eight years of chronological age to evaluate the global developmental quotient (GDQ) and *Wechsler Intelligence* Scale *for Children - III* [35] for children between six and sixteen for global intellectual quotient (GIQ). Wechsler Preschool and Primary Scale of Intelligence – 3rd edition was used in one patient. The normal range of GDQ and GIQ is on average (100) ± 1 SD (15).

The classification of the intellectual disability rank based on the GDQ/GIQ levels distinguishes four categories: *mild* intellectual functioning (IQ level: 50-69), *moderate* ID (IQ level: 35-49), *severe* ID (IQ level: 34-20) and *profound* ID (IQ level: below 20) [ICD-10]. Children with an GIQ/GDQ between 70 to 85 with learning disabilities were also considered in this study as borderline GIQ level.

The *Conners Parent and Teacher Rating Scales* [36] was used as a screening tool for attention deficit hyperactivity disorder (ADHD).

ETIOLOGIC INVESTIGATION

LABORATORIAL INVESTIGATION

All patients were submitted to blood and urine tests such as plasma amino acids, carbohydrate-deficient transferrin, urine amino acids, organic acids, guanidinoacetic acid and creatine to rule out metabolic diseases.

OTHER SUPPLEMENTARY DIAGNOSTIC TESTS

Neuroimaging studies, as cranio-encephalic MRI, were performed in the presence of microcephaly/macrocephaly, skin or dysmorphic features as well as motor neurologic signs.

GENETIC INVESTIGATION

All the patients included in the study were tested for the routinely performed laboratory tests such as, high-resolution G-banding karyotype, FISH studies when specific syndrome was suspected and fragile X testing when clinically indicated (FRAXA and FRAXE).

Forty two patients with idiopathic ID were eligible for array-CGH study. Patient´s and parents DNA was collected at HP and was sent to Life and Health Science Research Institute (ICV´s) in Minho´s University.

ARRAY-CGH

DNA extraction

Genomic DNA was extracted from peripheral blood using the Citogene® DNA isolation kit (Citomed, Portugal) according to the manufacturer´s protocol (http://www.citomed.com/index.php?option=com_content&view=article&id=46&Itemid=63).

Oligonucleotide array-CGH

The aCGH analysis was performed on a human genome CGH Agilent 180K custom array designed by the *Low Lands Consortium* (*LLC*, Professor Klass Kok) in order to be

used in the analysis of children with ID/ development disorder (DD) (AMADID:023363; Agilent, Santa Clara, CA). The array contained about 180.000 in situ synthesized 60-mer oligonucleotide probes with a mean resolution of approximately one probe every 17Kb (the resolution can reach 11Kb in the RefSeq genes rich regions) designed especially for the study of neurodevelopment and/or congenital abnormalities associated disorders (probes enrichment in regions known to be associated with DD/ID spectrum disorders). In the lower resolution areas, the array is guaranteed to detect at least CNVs above 75Kb. DNA labeling was performed using the ENZO Labeling Kit for Oligo Arrays (Enzo Life Sciences, Inc.).

Reference DNA (Kreatech Diagnostics), a homogeneous DNA pool from male or female human genomic DNA which has been isolated from 100 different anonymous healthy individuals. The ethnic background of the DNA poll is 85% of Caucasian and Hispanic and 15% of Afro-American. This product was specifically developed as areference DNA for array-CGH use. Arrays were then hybridized using the Agilent SurePint G3 Human CGH Microarray Kit. Briefly, samples and controls were hybridized in the presence of Cot-1 DNA and blocking agents for 24 hours, 65ºC and 20rpm. After the hybridization period, the slides were washed and scanned with Agilent Microrray Scanner. Data was extracted with the Agilent Feature Extraction (FE) Software v10.5 using default settings for CGH hybridizations.

Data analysis

Image analysis was performed using the across-array methodology described in the study [37]. CGH data was analyzed using Nexus Copy Number 5.0 software with FASST Segmentation algorithm and a minimum of three probes in a region required to be considered a copy number alteration. The FASST Segmentation algorithm is a novel Hidden Markov Model (HMM) based approach that, unlike other HMM methods, does not aim to estimate the copy number state at each probe but uses many states to cover possibilities, such as mosaic events, and then make calls based on a second level threshold. The stipulated minimal thresholds for calling a copy number gain were 0.2 (Copy number Gain) and 0.6 (High Copy Gain) and for calling a copy number loss were -0.2 (Copy number Loss) and -1 (Homozygous Copy Loss). The arrays design, database consultation and comparative analysis was performed using genome build 36.1/HG18.

Interpretation of CNVs

Interpretation of the CNVs found was carried out based on the workflow in figure 1. For each patient the total number of CNVs was listed according to the position in the chromosome and classified according to the workflow represented in figure 1.

Web resources

The following web resources were used for the interpretation of CNVs significance in the patients: Database of Genomic Variants (http://projects.tcag.ca/variation/), used for search of CNV described in healthy controls; University of California Santa Cruz (UCSC) Genome Bioinformatics (http://genome.ucsc.edu/), used for upload and visualization of "house-detected" CNVs databases; DECIPHER (https://decipher.sanger.ac.uk/application/), used for search of clinically significant CNVs; Online Mendelian Inheritance in Man (OMIM) (http://www.ncbi.nlm.nih.gov/omim) used for gene and clinical associated data search; PUBMED (http://www.ncbi.nlm.nih.gov/pubmed/), for bibliographic information search.

Gene Prioritization

The prioritization of the genes discussed for each patient was based on the data regarding its function described in OMIM and PubMed databases. Informatics analysis was not performed in these six patients.

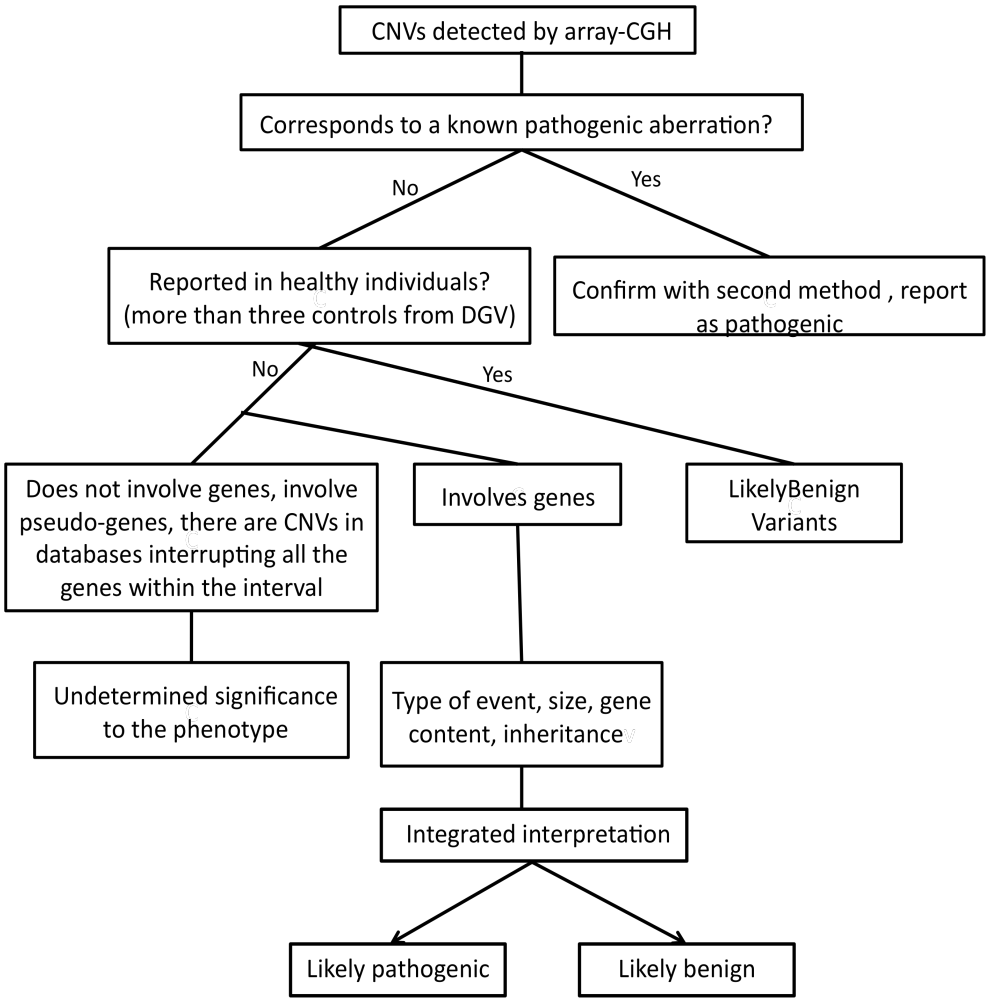


Figure 1. Schematic representation of the workflow followed for the interpretation of the CNVs found in each patient. (adapted from [38]).

RESULTS

The study sample from HP is composed predominantly of boys (57%). Thirty-five children (83,3%) had borderline or mild GIQ levels and only one (2%) had moderate GIQ levels. Only a minority of the patients (19%) had dysmorphic features (external years abnormalities, hipo and hipertelorism, skin abnormalities). Seven patients revealed some minor neurological alterations, such as abnormal walking in two patients (5%) and strabismus in four patients (10%) . There is a relevant personal history in 13 patients (31%), such as febrile seizures in four patients (10%), epilepsy in two patients (5%) and sensorial deficits in seven patients (17%). Family history of ID, learning problems during childhood or other psychopathologies, such as depression and epilepsy, was present in 25 families out of the 42 (60%). However, in the cases of positive history for ID, only one family was formally assessed until now. The clinical characteristics of the sample of children provided by HP are summarized in Table 2. The clinical findings of the 6 patients that will be discussed in detail are exposed in Table 3.

After array-CGH, many alterations in each of the six patients were found, with a maximum of 54 in patient three and a minimum of 14 in patient four (Appendix 2, Table S1-6) . However, the majority of these chromosomal aberrations detected were suspected to be polymorphic loci as there were CNVs reported in more than 3 controls (Database of Genomic Variants and UCSC Genome Bioinformatics). Alterations for which there were smaller CNVs described in the controls interrupting all the genes, were considered of unknown clinical significance. Alterations that did not include genes, that included pseudo-genes or with genes that are not expressed in central nervous system (CNS) were also considered as unknown clinical significance (Annex 2, Tables S1-S6, *green*). For the remaining alterations, further investigation was necessary in order to clarify their relevance to the phenotype, namely type of event, size, gene content and functions and inheritance pattern (Appendix 2, Tables S1-S6, *red*; Table S7), as well as environmental factors.

Table 2. Clinical characteristics of the 42 patients with unexplained ID.

Characteristic	Value
Sex:	
Male	24
Female	18
Age group (years):	
Median	10
Range	3-17
Age of diagnostic (years)[1]:	
Median	6
Range	1-15
Level of ID[2]:	
Borderline (70-85)	17
Mild (35-49)	18
Moderate (34-20)	1
Severe (<20)	0
Dysmorphic features:	
Yes	8
No	34
Growth abnormalities (Head circumference and/or stature)**:**	
Yes	6
No	36
Neurological exam:	
Normal	35
Clumsy	7
Personal history (pre and peri-natal problems, sensorial deficits, epilepsy)	
Yes	13
No	29
Family history (ID/learning problems and/or psychopathologies)**:**	
Yes	24
No	18
Consanguinity:	
Yes	1 (2nd degree)
No	42

[1]Not known in 7 cases / [2]Missing in 6 cases

Table 3. Clinical characteristics of the six patients discussed in detail.

Case/gender /current age	GIQ/ GDQ	Dysmorphisms	Growth parameters	Neurologic findings	Other findings	Familial history of cognitive impairment
1/M/15	67[1]	Auricular tags and pits	At birth: • W-3340 (P60) • H-48,5 (P40) • HC-34 (P50)	-	-	Mother[4]: + Father[4]:- Sister[4]: +
2/F/11	67[1]	Thin skin	At birth: • W-3740 (>P90) • H-52 (>P90) • HC-35 (P75)			Unknown
3/M/9	61[1]	One hiperchromic spot ("*café au lait*") Thin skin	At birth: • W-3150 (P50) • H-47,5 (P20) • HC-34 (P50) BMI(8years old) • >2SD		ADHD	Mother[5]: + Father[5]: - Sister:
4/M/7	74[2]	Thin skin Thin hair Unusual hair whorl	At birth: • W-3330 (P75) • H-49,5 (P50) • HC-32 (P15)	Tiptoe walking		Mother[4]: + Father[4]:- Brother[4]: +
5/M/8	82[3]	"Peculiar face"	At birth: • W-3530 (P75) • H-50,5 (P90) • HC-35 (P75) BMI(8years old) • <3SD		Poor weight gain since 3 months old Eating problems	Mother[4]: + Father[4]:- Sister[5]: +
6/M/11	50[1]	Prominent ears	At birth: • W-3850 (>P90) • H-52 (P75) • HC-35 (P75) BMI(11years old) • >2SD		ADHD/ Oppositional defiant disorder	

M – male; F- female; W (g) –weight; H (cm) – height; HC (cm) – head circumference / BMI – body mass index / ADHD – attention deficit and hyperactivity disorder; + positive family history; - negative family history

[1]*Wechsler Intelligence* Scale *for Children - III* / [2] *Ruth Griffiths Developmental Scale* / [3]*Wechsler Preschool and Primary Scale of Intelligence*

[4]Not formally assessed / [5] Formally assessed

CLINICAL REPORTS AND DISCUSSION

Patient 1

This is a 15 year old boy with mild ID (GIQ=67) diagnosed at the age of 8. There were no significant overall delay of psychomotor development in the first years of life, walking with 14 months, speaking first words with 18 months, and achieved toilette training with 20-22 months. He has minor dysmorphic facial features, namely auricular tags and pits. The remaining physical, neurological and sensorial evaluation was normal.

Pregnancy was monitored and uneventful. There is no record of teratogenic exposure. He was born through vaginal term delivery, without any complications. Apgar scores were normal (1st minute 9 and 5th minute 10) and no resuscitation was required. Growth parameters at birth were also normal (Table 3).

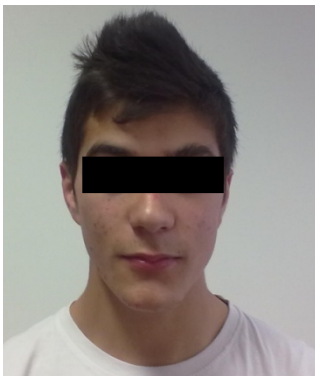


Figure 2. Facial appearance of patient 1.

The boy was born of non-consanguineous parents. His mother has epilepsy and depressive disorder, and his sister also has epilepsy and developmental delay. The father was described as a cognitively person, however, no formal cognitive assessment was performed.

Array-CGH revealed one CNV that required further investigation among 17 more alterations considered as benign variants (Appendix 2, Table S1) – a paternally inherited duplication at chromosome region 13q14.3, containing three genes (Appendix 2, Table S7).

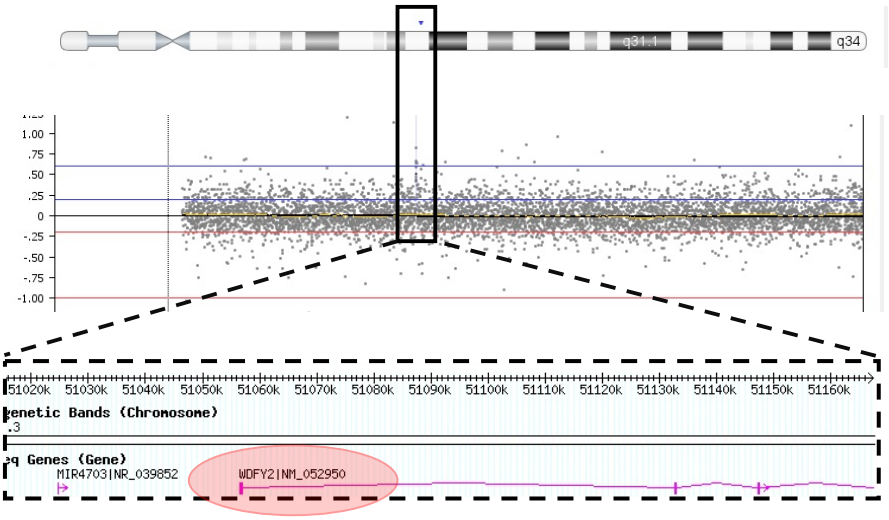


Figure 3. Schematic representation of the genomic alteration (13q14.3 duplication) and of the gene involved.

The 13q14.3 duplication covers, among others, the *WDFY2* (wd repeat- and fyve domain-containing protein 2) gene. WD repeat proteins have critical roles in many essential biological functions ranging from signal transduction, transcription regulation, to apoptosis, but is also recognized by their association with several human diseases [39] [. A study [40] identified a novel propeller-FYVE protein, ProF, which consists of seven WD-repeats and a FYVE domain. WD-repeat proteins offer a platform for protein-protein interactions by folding into a seven-bladed propeller-like structure, while the FYVE domain binds to phosphatidylinositol 3-phosphate present mainly on intracellular membranes. The results suggest that ProF plays a role in association with vesicles and could be involved in numerous vesicular processes in brain and pancreas, where its expression levels are comparatively high. The *WDFY2* is not being disrupted, even if the duplication is located in tandem with the original locus, but there is the possibility that this gene is sensitive to the dosage.

The search in Decipher database did not reveal any patient with a similar alteration, which did not allow to draw any conclusions through the comparison of similar genetic alterations and their respective phenotypic presentation. Most of the alterations documented on Decipher database for this cytoband are deletions and bigger alterations and, consequently, for which more noxious effect on phenotypic expression is

expected.It is also important to point out that this alteration was inherited from a reportedly normal father, but as mentioned before, no formal clinical assessment was performed.

Patient 2

This patient is a 11 year old girl with mild ID (GIQ=67) diagnosed when she was 8 years old due to specific school learning impairment. She had no relevant developmental delay in her early years. She spoke her first words at the age of 18 months and the first sentences when she was 28 months old. Physical examination revealed dark and thin skin. There were no other relevant clinical findings.

She was born after a monitored pregnancy with no complications. The delivery, at 39 weeks of gestation, had no complications as well. Apgar scores were 9 at 1st minute and 10 at 5th minute. She had adequate growth parameters at birth for her gestacional age. There is no history of alcohol or tobacco abuse or other teratogenic exposure during pregnancy.

She was born of unrelated parents. The mother is from the Dominican Republic and has similar physical characteristics with the girl, including skin tone and facial features. However, no psychometric assessment was performed. The father had reference of learning difficulties in childhood.

Figure 4. Facial appearance of patient 2.

Array-CGH revealed fifteen alterations (Appendix 2, Table S2), but most were considered benign CNVs respecting the criteria previously presented. In this case there

were not found clinically significant findings neither relevant genetic alterations after array-CGH that might explain the girl´s ID.

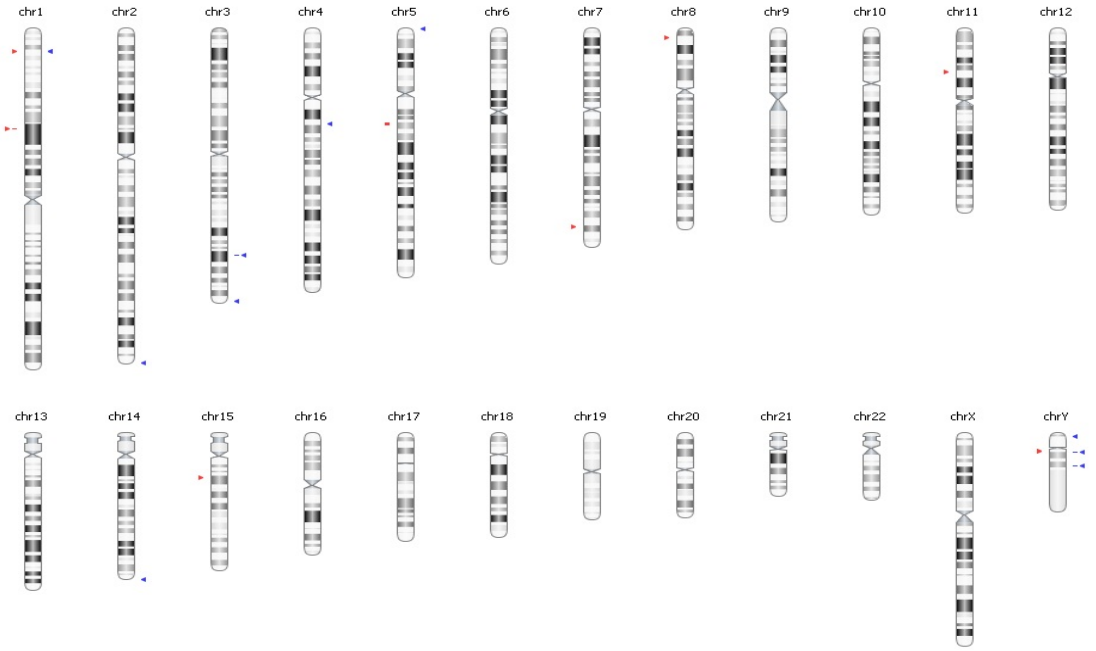


Figure 3. Overview of the alterations found by array-CGH on patient 2 (**red** –deletions / **blue** – duplications).

Patient 3

Patient 3 is a 9 year old male child diagnosed with mild ID (GIQ=61) at the age of seven. Developmental milestones were achieved in the normal range for his age, speaking the first words at 12 months, first sentences at 24 and achieving toilette training at 24 months too. He was also diagnosed with ADHD, being treated with methylphenidate in the past. Posterior physical examination unveiled abnormal skin texture (thin texture) and skin pigment alteration (one *café-au-lait spot*), but no significant dysmorphic facial features were found. The boy is obese, with a body mass index (BMI) of 23,76kg/m^2 (>2SD).

Pregnancy proceeded without complications and without teratogenic exposure. Birth was through vaginal delivery at 40 weeks of gestation, without any complications. No resuscitation was required, but Apgar scores are unknown. He had adequate growth parameters at birth for his gestacional age.

He is the second child of non-consanguineous parents. The mother was submitted to formal cognitive evaluation, which revealed cognitive impairment, despite being a functional person in daily live activities. Even though there are clinical indicators of cognitive impairment in his father and sister

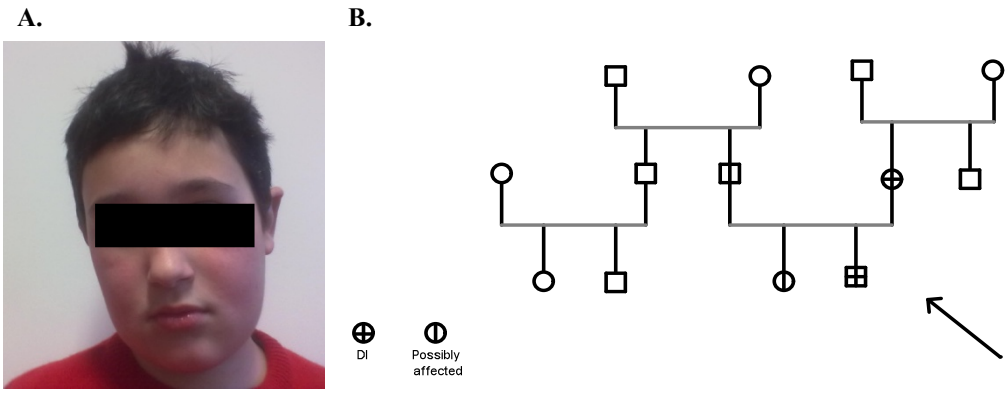


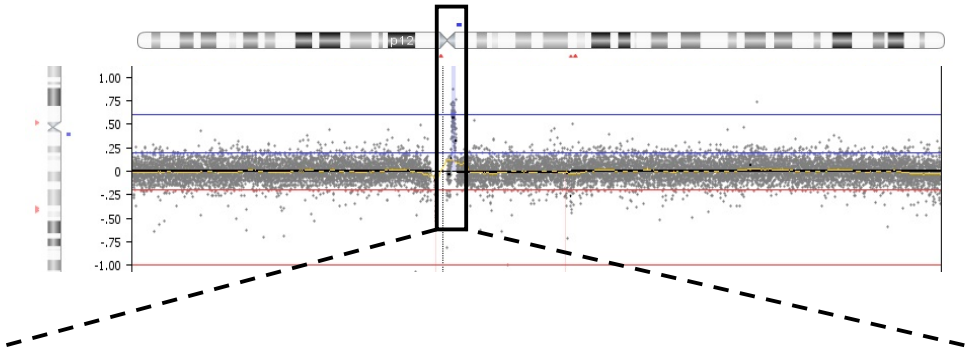
Figure 5. A- Facial appearance and **B-** pedigree representation for patient 3.

Array-CGH revealed a 1.5 Mb duplication at chromosome region 2q11.2 containing 24 genes (Appendix 2, Table S7).

Among the genes present in the duplicated region of patient 3, we can find some genes which seem to play a functional role in the brain. Among this, we can find *ADRA2B* (alpha-2b-adrenergic receptor), *ITPRIPL1* (inositol 1,4,5-triphosphate receptor interacting protein-like 1), *LMAN2L* (lectin mannose-binding 2-like protein), *SEMA4C* (semaphorin 4c) and *TMEM127* (transmembrane protein 127). *ADRAB2B* encodes an adrenergic receptor with high expression in liver and kidney. Alpha-2-adrenergic receptors have a critical role in regulating neurotransmitter release from sympathetic nerves and from adrenergic neurons in the central nervous system. *ITPRIPL1*does not have entrance in OMIM, but there is a study that reveals a protein-protein interaction network for human inherited ataxias and disorders of Purkinje cell degeneration [41]. The *LMAN2L* protein is highly expressed in kidney and skeletal muscle and it is also expressed in brain. This protein is thought to participate in the exportation of glycoproteins from the endoplasmic reticulum [42]. *SEMA4C* (semaphorin 4C) encodes a semaphorin. Semaphorins are a large family of secreted or membrane molecules that play diverse roles in axon guidance[43]. Several recent studies indicate an addicional role for semaphorins in synapse formation and stability [44]. A more recent study,

revealed that in addition to neuronal defects, the homozygous deletion of *SEMA4C* in mice also cause ventral skin pigmentation defects [45]. *TMEM127* encodes a transmembrane protein that localizes in the Golgi and lysosomes, suggesting that it may be involved in protein trafficking between these structures. Mutations in this gene also confers susceptibility for pheochromocytoma [46]. None of the genes mentioned are being disrupted and, therefore, an explanation for their effect on the child´s phenotype is the possibility of being dosage sensitive. However, the *SEM4AC* emerge as one of the most interesting genes in this region, and may be responsible for the child´s phenotype.

The alteration was inherited from a mother with cognitive impairment (formally assessed), which is in favor of the pathogenicity of this alteration. A similar alteration was previously reported [47] in a patient carrying a *de novo* 1.47 Mb duplication partially overlapping with this one. The phenotype of the patient reported consists in moderate ID, short stature, macrocephaly and hypotonia. Even with different inheritance patterns and with phenotypes that do not overlap completely, this region is probably the cause of this patient´s ID, due to the gene content and because it was inherited from an affected parent. The Decipher patient with the most similar alteration (#254924) has a smaller (0.9 Mb) duplication affecting partially the 2q11.2 cytoband. The alteration was inherited from a parent with similar phenotype to the child, yet no clinical information is provided.



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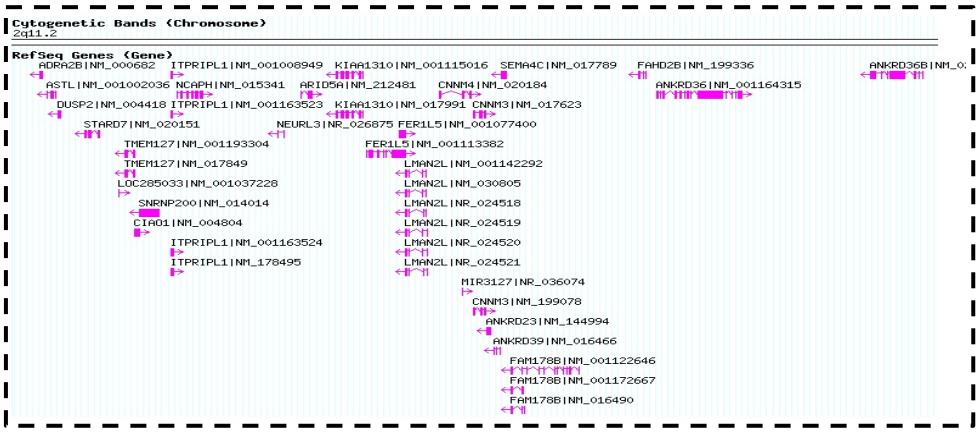


Figure 6. Schematic representation of the genomic alteration (2q11.2 duplication).

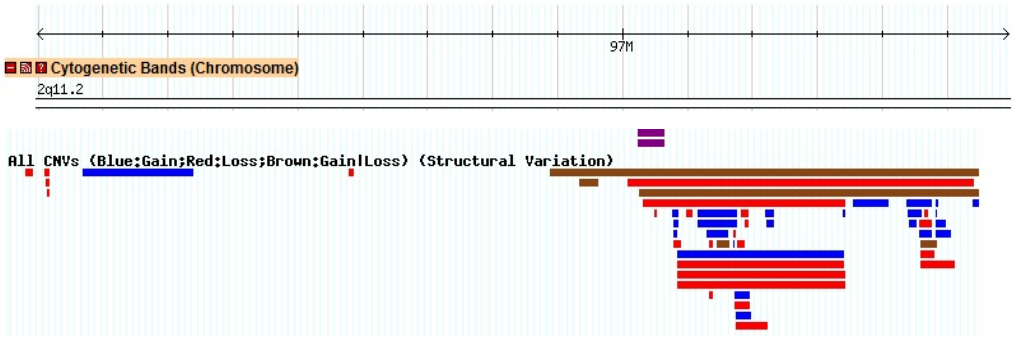


Figure 7. Schematic representation of the described CNVs in control in healthy individuals in DGV for the region 2q11.2 (blue lines for the duplications and red lines for the deletions).

Also in patient 3, array-CGH revealed a *de novo* duplication at chromosome region 7q31.33 containing one gene – *GPR37* (G protein-coupled receptor 37). G protein-coupled receptors (GPRs or GPCRs) contain 7 hydrophobic transmembrane domains connected by hydrophilic intra and extracellular loops. They transduce a variety of hormone, endogenous peptide, and neurotransmitter signals into intracellular effects via G proteins. A study [48] identified the protein encoded by *GPR37*, which is called PAELR (parkin-associated endothelin receptor-like receptor), as a protein that interacts with parkin, as a protein responsible for autosomal recessive juvenile Parkinson disease (PDJ). When overexpressed in cells, PAELR tends to become unfolded, insoluble, and ubiquitinated. Parkin specifically ubiquitinates PAELR in the presence of ubiquitin-conjugating enzymes resident in the endoplasmic reticulum (ER) of dopaminergic neurons and promotes the degradation of insoluble PAELR, resulting ER stress leading to neurodegeneration. Moreover, it was showed [48] that the insoluble form of PAELR

accumulates in the brains of PDJ patients, concluding that unfolded PAELR is a substrate of parkin and that the accumulation of PAELR may cause selective neuronal death in PDJ. The limit of the duplicated region of our patient is in the middle of the *GPR37*gene, meaning that if located in tandem the duplication can lead to its disruption and, consequently, altered function.

The decipher database did not show any similar alteration to the 7q31.33 duplication revealed in this case.

It is not known yet if this alteration is present or not in the parents. If the alteration reveal to be present in the mother (low IQ), we can infer that this mutation is likely to be pathogenic, but if it reveal to be inherited from the father (normal IQ) is more likely to be a benign variant.

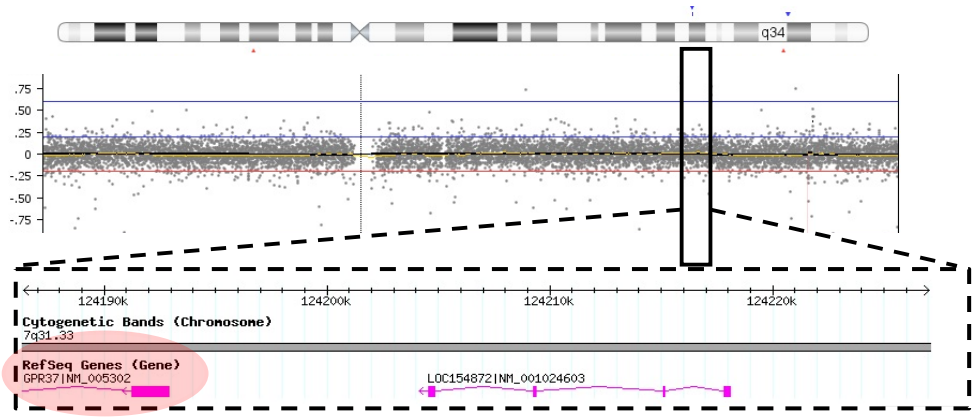


Figure 8. Schematic representation of the genomic alteration (7q31.33 duplication).

Patient 4

This is a male patient with seven years of age, diagnosed with a DD at the age of 4. As a child, his speech and language development were delayed, speaking the first words at the age of 24 months and first sentences at the age of 36 months. Motor development was normal for his age. At the age of 6 it was was diagnosed with borderline ID (IQ=74). Physical examination revealed tiptoe walking, thin skin, thin hair and unusual

hair whorl. He has low percentile (P15) for head circumference, since birth, as does his mother. No other growth anomalies were detected.

During the pregnancy, the mother was diagnosed with maternal hypertension which was treated with anti-hypertensive drugs (not specified). Birth was by normal vaginal delivery at 38 weeks of gestation and no resuscitation was required. The weight and height at birth were adequate for the gestacional age.

Positive history for learning disorder is present within the family. His mother and brother were diagnosed with dyslexia..

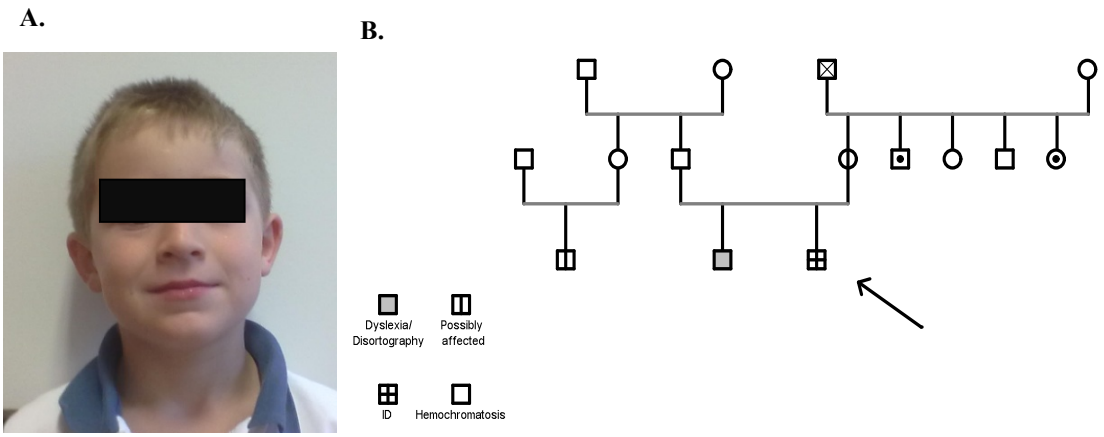


Figure 9. A- Facial appearance and **B-** pedigree representation for patient 4.

Array-CGH revealed three aberrations (out of 14) that needed further investigation: a 0,18 Mb paternally inherited duplication in cytoband 5q11.2, a 0,25Mb deletions 18q12.1 also inherited from the father and a *de novo* 0,98 Mb duplication in the cytoband 20q11.3 (Appendix 2, Table S7).

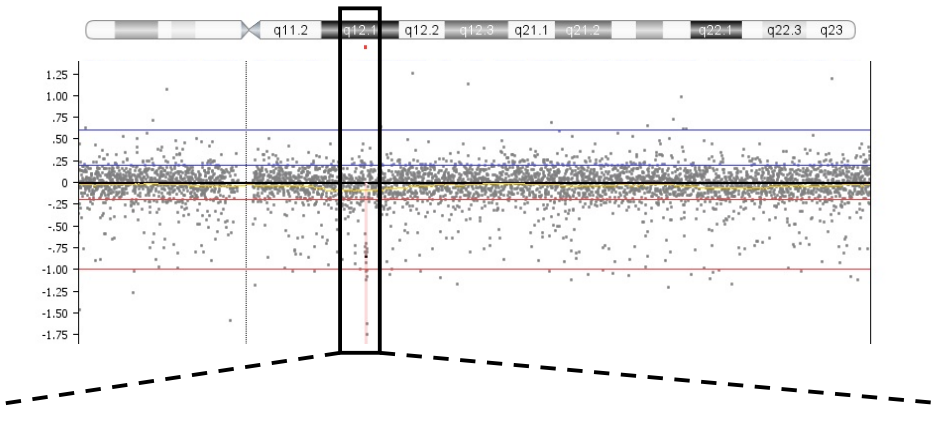
The duplication 5q11.2 contained only one gene: *ARL15*. It was associated to childhood obesity susceptibility However, our patient is not obese or overweighed, but as these variants are individually rare, it is unlikely that this alterations contribute greatly to the heritability of obesity. The Decipher patient with the most similar alteration (Decipher #248394) has a paternal 0,17 Mb duplication that partially overlaps with the one found in patient 4. Decipher #248394 displays a marked phenotype with multiple dysmorphisms, overlapping our patient´s phenotype only in ID. However, this Decipher

patient has another alteration 18,58 Mb deletion in chromosome 1 with 42 OMIM genes, which is more likely to explain his phenotype.

The deletion in cytoband 18q12.1 covers two genes: *MCART2* (mitochondrial carrier triple repeat 2), which has no entrance in OMIM or PubMed, and *KIAA1012*. *KIAA1012* is also called *TRAPPC8* (trafficking protein particle complex, subunit 8) and is a gene with highest expression in testis, spinal cord, and thalamus. *TRAPPC8* is a component of the *TRAPP* multisubunit tethering complex involved in intracellular vesicle trafficking. No similar alteration was found in Decipher database.

A *de novo* duplication at cytoband 20q11.23 with 0,09 Mb, containing two genes, which contains *VSTM2L* (V-set and transmembrane domain containing 2 like), is a candidate to explain his phenotype. This gene has no entrance in OMIM, but PubMed revealed a study [49], which describes *VSTM2L*, previously known as *C20orf102*, as a new modulator of neuroprotective activity of humanin (HN). HN is a 24-residue peptide displaying a protective activity in vitro against a range of cytotoxic and neurotoxic insults, as well as mediating in vivo amelioration of Alzheimer disease (AD)-related memory impairment in experimental models. *VSTM2L* is selectively expressed in the CNS in distinct brain areas as well such as primary cultured neurons, where it plays a role in the modulation of neuronal viability. Once more, there are no similar alterations in Decipher database.

In the three potential pathogenic duplications present in this child, the most interesting genes are not being disrupted by the duplicated region, but once more they cannot be ruled out as a possible cause of the phenotype.



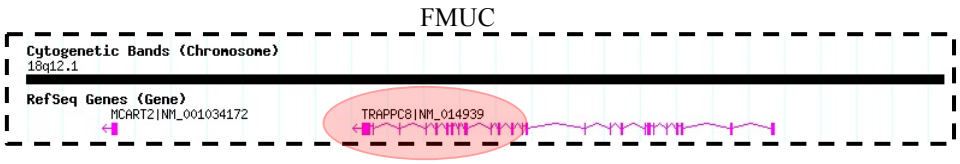


Figure 10. Schematic representation of the genomic alteration (18q12.1 deletion).

Patient 5

A eight year old boy with borderline ID (IQ=82) diagnosed at the age of 6 years old. He showed development delay since early months of life, sitting without support at 9 months, walking at 18 months, speaking his first words and sentences at 36 and 48 months. His BMI is 12,2kg/m^2 (<3SD). Physical examination did not show any dysmorphic features and the neurological exam was normal. There is history of developmental dysplasia of the hip (DDH), eating-related problems and poor weight gain in the first months of age.

Pregnancy was monitored and there is no record of complications. No teratogenic exposure was mentioned. Pre-natal growth was adequate and he was born by vaginal delivery without incidents, at 39 weeks of gestation. No resuscitation was required at birth.

The boy has a sister with ID (IQ=64) and epilepsy. Her physical examination was normal. Their mother had learning difficulties. No neurodevelopmental problems were reported in the father.

Array-CGH revealed 20 alterations and in four further analysis was necessary to conclude if they might be associated with our patient´s phenotype or not (Appendix 2, Table S7).

A. **B.**

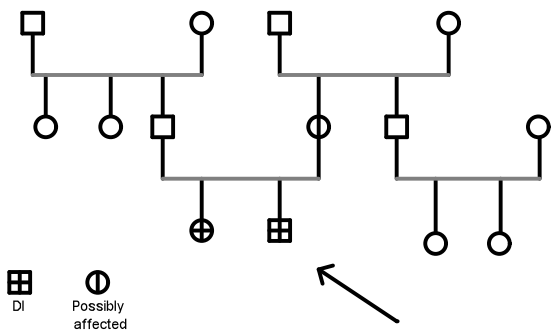


Figure 11. A- Facial appearance and **B-** pedigree representation for patient 5.

The deletion of cytoband 10q23.3 contains five genes (Appendix 2, Table S7). No useful information regarding their potential involvement on the patient´s phenotype was found. Besides that this deletion was inherited from a reportedly "normal" father.

The 22q13.33 duplication contains five genes and one of these is a possible candidate to explain the phenotype for our patient. The *ALG12* gene (homolog of asparagine-linked glycosylation 12 or dolichyl-p-mannose:Man-7-GlcNAc-2-PP-dolichyl-alpha-6-mannosyltransferase) encodes an (enzyme dolichyl-P-mannose:Man-7-GlcNAc-2-PP-dolichyl-alpha-6-mannosyltransferase) that catalyzes the addition of the eighth mannose residue onto the lipid-linked oligosaccharide precursor (LLO) during the synthesis of complex oligosaccharide-linked glycoproteins, which takes place in the endoplasmic reticulum and Golgi apparatus. A homozygous mutation in the human ALG12 gene was identified in a newborn who presented with failure to thrive, hypotonia, facial dysmorphism, progressive microcephaly and who showed hypoglycosylation of serum transferrin characteristic of congenital disorder of glycosylation type Ig (CDG Ig) [50]. Both parents were found to be heterozygous for the mutation and clinically normal. In a 2.5-year-old boy with CDG Ig who presented with psychomotor retardation, hypotonia, growth retardation, dysmorphic features and anorexia, compound heterozygosity for mutations in the ALG12 gene was identified [51]. The duplicated region does not disrupt the gene, even if located in tandem, and even if it did it would be in heterozygosity. No similar alteration was found in Decipher database

The 22q13 duplication is not common, with only a few cases reported. Some clinical features, including growth delay, microcephaly, congenital heart disease, facial dysmorphisms were described in a study [31]. Although our patient shares a similar feature to the one reported (including growth delay), the latter one has 7 different genes affected within this region, including SHANK3.

Array-CGH also revealed a 0,2 Mb maternally inherited duplication at chromosome region Xp11.2 which covers, among other genes, the *HUWE1* (E3 ubiquitin ligase) gene. This gene was associated to ID in a study [52], where they identified six different but overllaping microduplications at Xp11.22. Patients were non-syndromic with mild

to moderate ID. The proposed causal role for this duplication comes from the fact that all segregated with the disease in the six described families and it was never found in controls. Moreover, mutations in several proteins involved in ubiquitin pathway have been related to ID [53-55] and dysfunction of the ubiquitin metabolism is a hallmark in several neurological degenerative diseases [56-57]. Decipher database revealed a patient (#4484) with a similar alteration inherited from normal parent that partially overlaps with our child. As our patient, #4484 has ID and no more clinical features are described.

Along with the other alterations, patient 5 also has a *de novo* duplication of cytoband Xq28. The duplication of 0,15 Mb affects eight genes (Table 10). *L1CAM* (L1 cell adhesion molecule) is a good candidate to his phenotype. The L1 cell adhesion molecule is one of a subgroup of structurally related integral membrane glycoproteins belonging to a large class of immunoglobulin superfamily cell adhesion molecules (CAMs) that mediate cell-to-cell adhesion at the cell surface. *L1CAM* is found primarily in the nervous system of several species and may be more aptly called a neural recognition molecule [58]. The same study reviewed the various functions of L1CAM, including guidance of neurite outgrowth in development, neuronal cell migration, axon bundling, synaptogenesis, myelination, neuronal cell survival, and long-term potentiation. Loss of function mutations in the L1 gene are responsible for an X-linked recessive neurological disorder that has been described as X-linked hydrocephalus, MASA syndrome or spastic paraplegia type I [58]. At Decipher database we did not find any similar alteration

Among all the alterations, the most promising in this patient is the Xp11.22 duplication. It is known that the mother had some learning difficulties during childhood, but her current cognitive level was not evaluated. This kind of qualitative information is difficult to take into account. The mother can have a "*marginal*" phenotype or, on the hand, be a non-manifesting carrier. Studies of X-inactivation may be informative. It may also be important to seek other male relatives clinical history in the maternal family.

Patient 6

This 11 year old boy, sixth child of non-consanguineous Caucasian parents when the mother was 36 and the father was 38 years old, was diagnosed with mild ID (IQ=50) at

the age of 11 years old. The boy also has ADHD and myopia. Physical examination revealed only one minor dysmorphic facial feature (prominent ears). He has a normal neurological exam. His BMI is 25,4 kg/m² (>2SD) at the age of eleven, which corresponds to obesity.

Pregnancy and delivery progressed without any problems. Born by eutocic delivery at 38 weeks of gestation. There was a good adaptation to extrauterine life. The growth parameters at birth were normal (Table 3).

There is positive familial history from both sides (Fig. 10, C), but no formal evaluation was made yet. In the family there is also history of neo-natal death, with one sibling dying when he was 6 months old and 2 other siblings dying before reaching that age. The cause of those deaths are unknown.

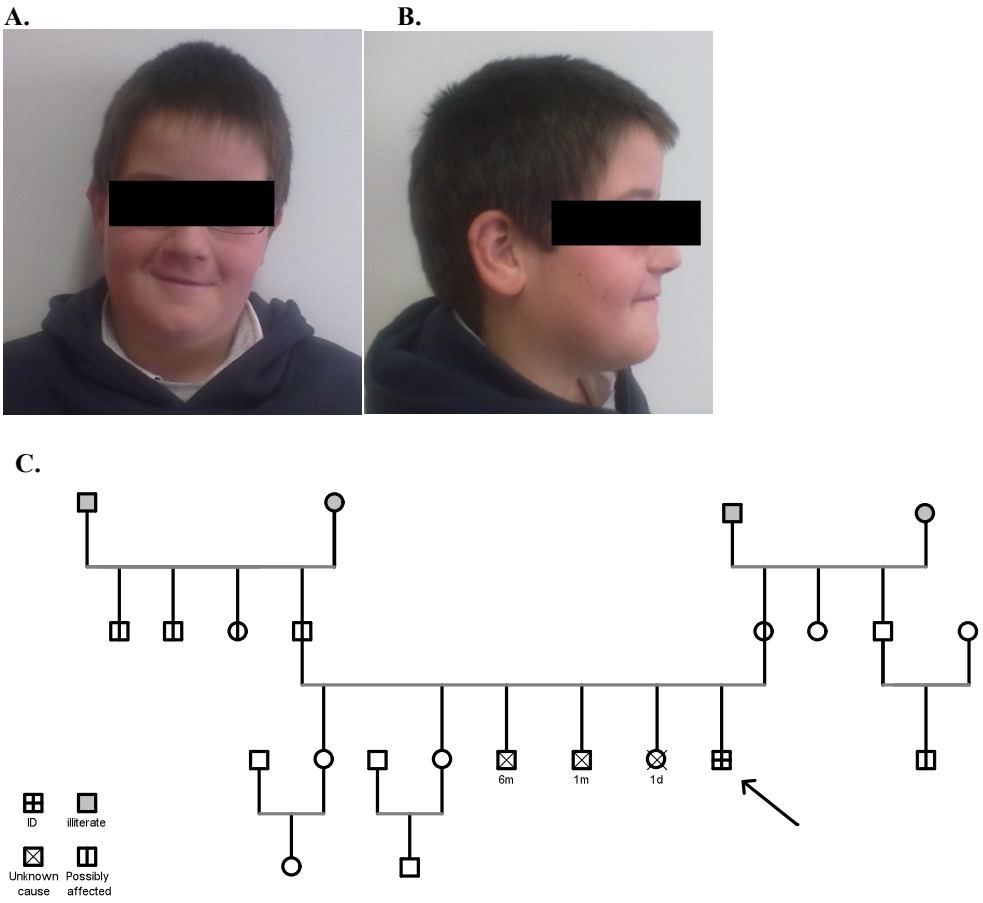


Figure 12. A/B- Facial appearance and **C-** pedigree representation for patient 6.

Array-CGH revealed 38 alterations, but the majority were considered benign variants, as they represented a common polymorphism in DGV. Four aberrations may putatively be the cause of the child´s phenotype, but further investigation was required as regards to size, gene content, inheritance pattern (Appendix 2, Table S7).

The duplication at chromosome region 9p13-p11.1 contains 58 genes, the majority not yet been studied/confirmed biologically. Among the affected genes, *CNTNAP3* (contactin-associated protein-like 3) may constitute a good candidate for the ID of our patient. *CNTNAP3* belongs to a subgroup of the neurexin family of multidomain transmembrane proteins that are involved in cell adhesion and intercellular communication [59]. Cell adhesion molecules are critical for the maintenance of synaptic structure and neuronal plasticity [60]. *CNTNAP3*, also called *KIAA1714*, show features of a cell adhesion protein. It shows intermediate expression in whole adult brain, kidney, and spinal cord, and in fetal liver and brain. Within the brain, highest expression was found in cerebellum, caudate nucleus, hippocampus, and substantia nigra. Another gene in the region is *ZNF68* (zinc finger protein 68). Although, no entrance was found in OMIM or Pubmed for *ZNF68*, a number of zinc finger proteins, autosomal and X-linked, have been implicated in NS-ID. It is possible that these proteins target the regulation of specific neuronal genes that are involved in cognitive development, learning or memory formation, resulting in NS-ID [7,8,23, 28].

A second alteration on chromosome 9 (9q12-q13 duplication) was revealed. This is also a gene-rich sequence. One of the genes in the region is PGM5 (phosphoglucomutase 5). Phosphoglucomutases, such as PGM5, are phosphotransferases involved in interconversion of glucose-1-phosphate and glucose-6-phosphate. PGM activity is essential in formation of carbohydrates from glucose-6-phosphate and in the formation of glucose-6-phosphate from galactose and glycogen [61], but no function in the CNS was described so far.

The first two duplications occurred at the centromeric region of chromosome 9 (9p13-p11.1 and 9q12-q13 regions). This is a highly polymorphic region, but no CNVs as large as the ones present in our patients were found. Both alterations were found in the mother, who is suspected to have cognitive impairment.

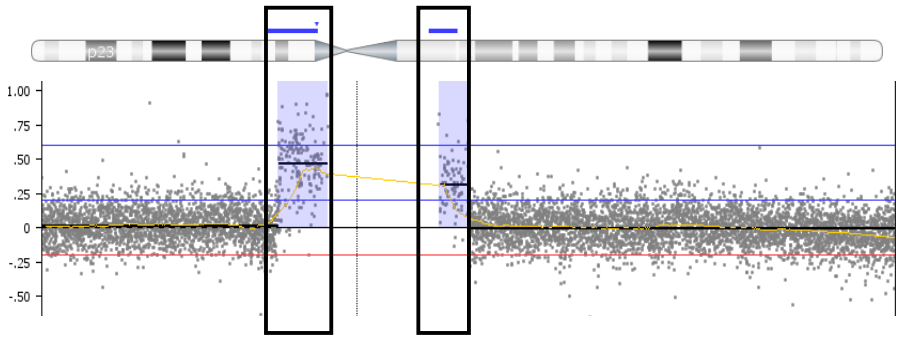


Figure 13. Schematic representation of the genomic alteration (7q13.33 duplication) (patient).

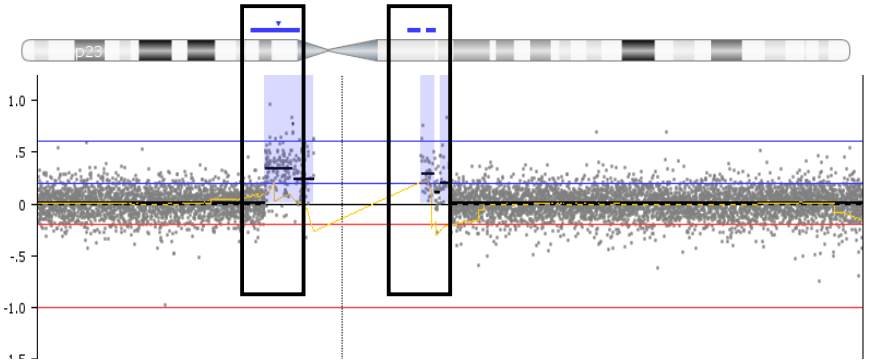


Figure 14. Schematic representation of the genomic alteration (7q13.33 duplication) (mother).

Another alteration found in patient 6 was a 1 Mb paternally inherited duplication at chromosome region 11p15.1. From the 65 genes in the region, four may be involved in the pathogenecity. Indeed, four of these genes (*DelGEF, HPS5, KCN1,TPH1*) are expressed in the brain and *TPH1* (tryptophan hydroxylase 1) is the most likely associated to the phenotype. Tryptophan hydroxylase catalyzes the biopterin-dependent monooxygenation of tryptophan to 5-hydroxytryptophan (5HT), which is subsequently decarboxylated to form the neurotransmitter serotonin. It is the rate-limiting enzyme in the biosynthesis of serotonin. *TPH* expression is limited to a few specialized tissues, including nervous system and has been associated to ADHD [62], suicidal behavior [63-64] and schizophrenia [65]. *KCNC1* (potassium channel, voltage-gated, shaw-related subfamily, member 1) encodes the subunits for the fast-activating/fast-deactivating, voltage-gated potassium channels Kv3.1 [66]. Kv3.1 and Kv3.2 are widely expressed in the brain, including areas implicated in the control of motor activity and in areas thought to regulate arousal states. It was showed [67] that double mutant mice (Kv3.1

and Kv3.3) display a "stronger" mutant phenotype that includes motor dysfunction (ataxia, myoclonus, tremor) and hyperactivity, when comparing to single mutant mice (Kv3.1) that showed increasing of stereotypic activity in conjunction with sleep loss. Search in Decipher database did not show any similar alteration.

Another alteration found with array-CGH was a 0,5 Mb duplication at chromosome region 20q13.3, containing twenty-nine genes. The OMIM search for the genes covered by the duplication revealed, among others, some interesting candidates, listed on Table S7 (Appendix 2). *CHRNA4* (cholinergic receptor, neuronal nicotinic, alpha polypeptide 4) is one of these genes. The nicotinic acetylcholine receptors (nAChRs) are members of a superfamily of ligand-gated ion channels that mediate fast signal transmission at synapses. *CHRNA4* mutations have been related autosomal dominant nocturnal frontal lobe epilepsy [68]. Regardless, these mutations in the *CHRNA4* gene appear to account for only a small proportion of the cases of nocturnal frontal lobe epilepsy. *KCNQ2* gene (potassium channel, voltage-gated, KQT-like subfamily, member 2) encodes a voltage-gated potassium channel that is expressed in the brain [69]. Mutations in this gene have been associated with benign familial neonatal seizures (BFNS) [70], which is a rare idiopathic, generalized epilepsy syndrome [71]. However, neither the patient or his mother have history of seizures. Moreover, a more recent study revealed that overactivation or expression of M channel induced apoptosis [72]. This investigation provided the first evidence that, in addition to their well-defined physiological functions, the activity of *KCNQ2/KCNQ3* channels may affect viability of central neurons, inducing neuronal cell death. Potassium –channel genes are also frequently involved in long QT syndromes (LQTS). As a result of these arrhythmias, patients suffer from recurrent syncope, seizures or sudden death as the most dramatic event. Mutations in five genes have been identified in LQTS [73], but there are no references relating to *KCNQ2* and LQTS. It may be possible that this mutation was the cause of death of the three siblings of patient 6. As LQTS are associated with premature sudden cardiac deaths affecting whole families [74], it would be important to study the rest of the family searching for other early deaths or cardiac problems, clarify his brothers' causes of death and screen our patient for heart diseases and.

The Decipher patient with the most similar alteration (#250915) has a 1,64 Mb *de novo* 20q13.3 duplication partially overlapping with the one found in patient 6. Decipher

#250915 displays only one overlapping characteristic with our patient, which is ID, but has also others features. However, we should take into account that this patient has two more alterations documented on Decipher and one of them is a 7,4Mb *de novo* deletion, which is more likely to be pathogenic.

GENERAL DISCUSSION AND CONCLUSION

Many genetic aberrations have been identified in patients previously diagnosed with "idiopathic" ID and/or NS-ID [8, 22-23]. Although CNVs exists in approximately 12% of the entire genome in the normal population, rare CNVs have been implicated in ID [75]. In fact, the etiological diagnosis of NS-ID has evolved positively with the development of new diagnostic tools, such as array-CGH, but at the same time new challenges have emerged. These difficulties are essentially related with the interpretation of the alterations found, which needs to take into account innumerous variables, and with the innate heterogeneity of NS-ID genotypes.

In this study, array-CGH was used to investigate possible genetic causes for the patient´s idiopathic ID, specifically DNA copy number imbalances. In our sample of 6 NS-ID, we have found until now two alterations that are very likely to be pathogenic, a duplication (CNV gain) in 2q11.2 (patent 3) and Xp11.22 (patient 5) in two different families. Both alterations were previously reported [47, 52] and contain genes with compelling gene functions that could be relevant to the patient´s phenotype and have inheritance patterns that do not exclude them as pathogenic . On the other hand, in some alterations it was not so easy to infer, or to exclude, the clinical significance of these genomic imbalances on the phenotype expressed by the children.

The smallest alteration found was a 0,04 Mb 7q31.33 duplication in patient three. Despite being a small genomic imbalance it contains an interesting gene (*GPR37*). Besides that, the limit of the duplicated region is occurring inside this functional gene, which may, if located in tandem, cause its disruption by encoding a mutant protein that is degraded or has an altered function. This type of alterations may lead to loss or gain-of-function of a gene and represent one of the several mechanisms that can cause clinically significant alterations [76]. The 9p13-p11.1 and 9q12-q13 duplications in patient 6, on the contrary, were the largest aberrations found with a considerable size of 8Mb and 4,8Mb, respectively. Such bigger genomic imbalances are more likely to be pathogenic [27, 77]. Furthermore, CNVs as large as these ones were not reported until now. Therefore, they should not be excluded as non-pathogenic, although they are in a highly polymorphic region and the second alteration does not contain any gene with a compelling function. This example, regarding the size, shows that generalizations drawn

between CNV size and significance hold true as general rule, but they should not exclude CNV as clinically significant based only on that fact [33]. The same goes for the gene content. A CNV that is gene rich is more likely to be pathogenic comparing to a region which is void of genes or has pseudogenes [33]. However, recent studies show that these alterations can also play a role in the pathogenicity through gene fusion (between genes or their regulatory sequences), which can create a "*gain-of-function*" mutation and trough position effect by removing or altering a regulatory sequence, which can have an effect on expression or regulation of a nearby gene, for example [25]. Given that over 40% of all genes are expressed in human brain, CNVs might contribute significantly to cognitive, behavioral and psychological variation, pathological or not [23]. But when dysmorphic features are absent or there are few, narrowing down to a candidate gene as the cause of the phenotype is difficult. The challenging aspect of research on the genetic determinants of ID will be to understand the contribution of genes to learning disorder, mild ID and NS-ID. It is necessary to improve the knowledge about the pathways involved in NS-ID, which has been facilitated, recently, by the discovery of new genes as the cause of NS-ID. Some pathways are emerging as central contributors to normal cognition and the expectation is that this will enhance the efficiency for genetic diagnosis of NS-ID.

During the assessment of rare variants´ pathogenicity, besides the overlapping with known genomic imbalance (for example, a previously published syndromes), the size and the gene content, also the type of event (duplication *versus* deletion) and the inheritance pattern should be taken into account. Duplications are indeed supposed to be better tolerated in the genome than deletions and are more commonly observed in healthy individuals [78]. So, if the CNV is a duplication and it does not contain dosage-sensitive genes, this indicates that it is probably benign [27]. However, duplications of dosage-sensitive genes may also lead to diseases [79]. Dosage-sensitive genes are genes that, although are not being disrupted by the duplicated or deleted region, they have a known relation with the phenotype. Reduced gene dosage produces haploinsufficiency effect, which is commonly more deleterious than gene over-expression. Curiously, the two most likely aberrations found are duplications. One of them is on the X chromosome and recent observations suggest that duplications are quite common in XLID patients [18, 52]. In the six cases exposed, only two deletions were found (Table

10) and both of them were inherited from reportedly "*normal*" fathers. This rules in favor of a benign variant. However, mutations inherited from normal parents cannot always be ruled out as pathogenic. Some special considerations should be taken into account such as incomplete penetrance, variable expressivity, imprinting and mosaicism [33]. All of these factors are critical when considering newly described deletions and duplication in a relatively small group of patients as this [47]. Alternatively, a deletion may be inherited from the unaffected carrier parent exposing a recessive mutation in the other allele. The same happens when a CNV is a d*e novo* mutation. This is a usually taken as evidence supporting pathogenicity [33, 38, 77], particularly if the CNV is suspected to have clinical significance based on other lines of evidence, such as gene content. However, interpretation is more difficult when the alterations are familial and it is not clear if there are phenotypic abnormalities in the family [23]. Patient 5 is the only one with *de novo* mutations and in the duplicated region (Xq28) there is a very compelling gene, which makes this alteration likely pathogenic (besides Xp11.22 duplication).

Moreover, it is important to consider other alterations present in those patients, which might modify the overall genetic expression and contribute to a more pronounced phenotype when comparing to their parents' phenotype. This additive effect of mutations should be considered, however evidence to support it is still lacking. Patient 6 is a good example of this fact and of the difficulties when interpreting CNVs. Similarly to other cases, we found duplications with potential genes, which are inherited from parents (Appendix 2, Table7) for whom cognitive impairment is suspected. Here we are playing with many variables, such as: duplications that do not interrupt the genes, which in order to have influence the phenotype they have to be dosage-sensitive; interesting genes that are expressed in the brain, and some related to neurological disorders, but are not known as ID genes yet; parents who are suspected to have cognitive impairment or behavioral problems, but were not formally confirmed.

When all the information necessary is not available, it is difficult to classify a CNV as likely pathogenic or *vice-versa*. Making assumptions about the pathogenicity of a CNV in cases like this is a risk and even if we had all the information we consider relevant, it would be only an assumption in some cases, at least for now. Nevertheless, this kind of information is still very important to establish the most correct correlation as possible. It highlights once again the importance of assessing parents and other members of the

family in order to detect undetermined or minimal clinical symptoms and the need to uncover more complex genetic mechanisms that could be behind human neurological development, behavioral and cognitive functioning. Additionally, knowing the origin of the abnormalities detected is essential to genetic counseling of affected families [80].As regards the interpretation of CNVs, gene-environment interactions should also be taken into consideration. Neurodevelopmental disorders are particularly likely to express these kind of interactions, because development itself is a dynamic process that results from a constant interplay between genetic and environmental determinants [81]. Having this in mind, new ways in the approach for people with ID should be drawn, especially in those cases where it is not possible to conclude with certainty a genetic cause, even with the most advanced technologies. An example of this can be patient 4. A *de novo* duplication (20q11.23) in this patient contains an interesting gene (*VSTM2L*). This gene modulates the neuroprotective activity of humanin against a range of neurotoxic insults. His mother had maternal hypertension during pregnancy and was treated with anti-hypertensive drugs. Some studies report the association between hypertension diseases of pregnancy and neurodevelopment outcomes in children [82-83]. These acquired environmental factors considered in association with a genetic susceptibility, for example a mutation of a gene that confers neuroprotection, can create a propitious field to abnormal development of the brain and result in a variety of outcomes based on the severity of both genetic and environmental "*aggressions*". In this line of thought, an accurate patient clinical history is very important, as well as consideration of other external factors such as family, social environment and schools, that can also modify the expected intellectual performance of children. This emphasizes the importance of a more dynamic and multidimensional understanding of the factors involved in phenotype expression such as genetic background, epigenetic factors and other modifier genes or environmental factors. Researching is moving in this direction and this kind of approach will be particularly important in the cases where learning disorders and ID is the main feature such as in our sample. In the particular case of autism, the absence of Mendelian inheritance patterns, meant that this was the first disease to be considered polygenic, i.e., a disorder caused by multiple genetic risk factors, each of weak effect. More recently, an multiple hit model was proposed that considered the autism as a group of

disorders caused by a heterogeneous genetic risk or protective interaction factors that influencing common neuronal pathways [84], a concept that may be expanded to ID.

Finally, it is worthwhile to discuss the cases where the alterations found were either considered not clinically relevant or with unknown clinical significance, as happened with patient 2. This kind of results can be due to limitations related to the technique or due to interpretation criteria established for CNV classification. The current limitations of array-CGH include the inability to detect balanced rearrangements [17, 38, 85]or regions consisting of highly repetitive sequences such as pericentromeric and heterochromatic regions as well as p-arms of acrocentric chromosomes [13]. Array-CGH does not detect alterations smaller than the distance between two probes, which corresponds to the resolution level (in our case 17 kb). The presence of *background noise* can also represent one limitation of the technique. Besides this, we considered most of the alterations found likely benign variants after comparison with controls from the DGV database and the database constituted by Dutch children with ID. Since, the controls we used are not Portuguese, their genetic background is probably different and this may lead to misinterpretations from the start. Then, there are the other alterations of unknown clinical significance such as: deletions/duplications for which CNVs have been described in control cases, but in less than three controls and/or not covering the entire region; genomic imbalances for which controls have a deletion described while the patient has duplication, and if the breakpoints affect the same genes, it is more likely that the duplication is not causing the disease, since deletions are more deleterious, as previously said; alterations that do not encompasses genes or contain genes for which biological function is still not known.

Ongoing studies, such as the one taking place in Portugal, can contribute to the discovery of new syndromes and candidate genes/regions underlying the condition, to elucidate new mechanisms involved in neural development, maturation and functioning and enable future correlations of genetic and clinical features, in researchers´ point of view. From clinicians´ point of view, this investigation will allow, in some cases, to establish a medical cause for these children´s ID, as well as family counseling and pre-natal diagnosis in a short-term perspective. It also enables the improvement of the educational and social response in future offspring.

Ultimately, we emphasize the importance of a detailed phenotypic description of the family in order to infer more accurate cause-effect relations between genotype and

phenotype. It is also important to underline that, with the increasing use of array-CGH in clinical practice, a closer interaction between clinicians and researchers is required. This will enable a more scrupulous interpretation and correlation of the genetic results with the patient´s clinical findings.

Despite all the limitations found, for two families it is now possibly to *"know"* the reason for their children´s ID and initiate new strategies of coping with the disease.

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REFERENCES

1. Ropers, H.H., *Genetics of intellectual disability.* Curr Opin Genet Dev, 2008. **18**(3): p. 241-50.
2. Salvador-Carulla, L. and M. Bertelli, *'Mental retardation' or 'intellectual disability': time for a conceptual change.* Psychopathology, 2008. **41**(1): p. 10-6.
3. Organization, W.H., *International Classification of Mental and Behavioural Disorders: Clinical description and diagnositic guidelines*, in *The ICD-10 Classification of Mental and Behavioural Disorders.* 1992. p. 1-267.
4. Leonard, H. and X. Wen, *The epidemiology of mental retardation: challenges and opportunities in the new millennium.* Ment Retard Dev Disabil Res Rev, 2002. **8**(3): p. 117-34.
5. Roeleveld, N., G.A. Zielhuis, and F. Gabreels, *The prevalence of mental retardation: a critical review of recent literature.* Dev Med Child Neurol, 1997. **39**(2): p. 125-32.
6. Chiurazzi, P. and B.A. Oostra, *Genetics of mental retardation.* Curr Opin Pediatr, 2000. **12**(6): p. 529-35.
7. Chelly, J., et al., *Genetics and pathophysiology of mental retardation.* Eur J Hum Genet, 2006. **14**(6): p. 701-13.
8. Kaufman, L., M. Ayub, and J.B. Vincent, *The genetic basis of non-syndromic intellectual disability: a review.* J Neurodev Disord, 2010. **2**(4): p. 182-209.
9. McLaren, J. and S.E. Bryson, *Review of recent epidemiological studies of mental retardation: prevalence, associated disorders, and etiology.* Am J Ment Retard, 1987. **92**(3): p. 243-54.
10. Froyen, G., et al., *Detection of genomic copy number changes in patients with idiopathic mental retardation by high-resolution X-array-CGH: important role for increased gene dosage of XLMR genes.* Hum Mutat, 2007. **28**(10): p. 1034-42.
11. Herbst, D.S. and J.R. Miller, *Nonspecific X-linked mental retardation II: the frequency in British Columbia.* Am J Med Genet, 1980. **7**(4): p. 461-9.
12. Bashi, J., *Effects of inbreeding on cognitive performance.* Nature, 1977. **266**(5601): p. 440-2.
13. Xu, J. and Z. Chen, *Advances in molecular cytogenetics for the evaluation of mental retardation.* Am J Med Genet C Semin Med Genet, 2003. **117C**(1): p. 15-24.
14. Qiao, Y., et al., *Outcome of array CGH analysis for 255 subjects with intellectual disability and search for candidate genes using bioinformatics.* Hum Genet, 2010. **128**(2): p. 179-94.
15. de Vries, B.B., et al., *Screening and diagnosis for the fragile X syndrome among the mentally retarded: an epidemiological and psychological survey. Collaborative Fragile X Study Group.* Am J Hum Genet, 1997. **61**(3): p. 660-7.

16. Hagberg, B. and M. Kyllerman, *Epidemiology of mental retardation--a Swedish survey.* Brain Dev, 1983. **5**(5): p. 441-9.

17. Sagoo, G.S., et al., *Array CGH in patients with learning disability (mental retardation) and congenital anomalies: updated systematic review and meta-analysis of 19 studies and 13,926 subjects.* Genet Med, 2009. **11**(3): p. 139-46.

18. Chiurazzi, P., et al., *XLMR genes: update 2007.* Eur J Hum Genet, 2008. **16**(4): p. 422-34.

19. Wu, Y., et al., *Submicroscopic subtelomeric aberrations in Chinese patients with unexplained developmental delay/mental retardation.* BMC Med Genet, 2010. **11**: p. 72.

20. Moeschler, J.B. and M. Shevell, *Clinical genetic evaluation of the child with mental retardation or developmental delays.* Pediatrics, 2006. **117**(6): p. 2304-16.

21. Ravnan, J.B., et al., *Subtelomere FISH analysis of 11 688 cases: an evaluation of the frequency and pattern of subtelomere rearrangements in individuals with developmental disabilities.* J Med Genet, 2006. **43**(6): p. 478-89.

22. Lee, J.A. and J.R. Lupski, *Genomic rearrangements and gene copy-number alterations as a cause of nervous system disorders.* Neuron, 2006. **52**(1): p. 103-21.

23. Stankiewicz, P. and A.L. Beaudet, *Use of array CGH in the evaluation of dysmorphology, malformations, developmental delay, and idiopathic mental retardation.* Curr Opin Genet Dev, 2007. **17**(3): p. 182-92.

24. Marshall, C.R., et al., *Structural variation of chromosomes in autism spectrum disorder.* Am J Hum Genet, 2008. **82**(2): p. 477-88.

25. Zhang, F., et al., *Copy number variation in human health, disease, and evolution.* Annu Rev Genomics Hum Genet, 2009. **10**: p. 451-81.

26. Aradhya, S., et al., *Whole-genome array-CGH identifies novel contiguous gene deletions and duplications associated with developmental delay, mental retardation, and dysmorphic features.* Am J Med Genet A, 2007. **143A**(13): p. 1431-41.

27. Miller, D.T., et al., *Consensus statement: chromosomal microarray is a first-tier clinical diagnostic test for individuals with developmental disabilities or congenital anomalies.* Am J Hum Genet, 2010. **86**(5): p. 749-64.

28. Bejjani, B.A. and L.G. Shaffer, *Clinical utility of contemporary molecular cytogenetics.* Annu Rev Genomics Hum Genet, 2008. **9**: p. 71-86.

29. Zhu, L., et al., *[Detection of the genetic abnormalities in patients with mental retardation using multiplex ligation-dependent probe amplification assay].* Zhonghua Yi Xue Yi Chuan Xue Za Zhi, 2009. **26**(6): p. 644-7.

30. Rooms, L., et al., *Array-based MLPA to detect recurrent copy number variations in patients with idiopathic mental retardation.* Am J Med Genet A, 2011. **155A**(2): p. 343-8.

31. Knight, S.J., et al., *Subtle chromosomal rearrangements in children with unexplained mental retardation.* Lancet, 1999. **354**(9191): p. 1676-81.

32. Kearney, H.M., et al., *American College of Medical Genetics recommendations for the design and performance expectations for clinical genomic copy number microarrays intended for use in the postnatal setting for detection of constitutional abnormalities.* Genet Med, 2011. **13**(7): p. 676-9.

33. Kearney, H.M., et al., *American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants.* Genet Med, 2011. **13**(7): p. 680-5.

34. Griffiths R., *The abilities of young children.*, London: University of London press, 1984.

35. Wechsler D, *Wechsler Intelligence Scale for Children,* New York: The psychological corporation,1949.

36. Goyette, C.H., C.K. Conners, and R.F. Ulrich, *Normative data on revised Conners Parent and Teacher Rating Scales.* J Abnorm Child Psychol, 1978. **6**(2): p. 221-36.

37. Buffart, T.E., et al., *Across array comparative genomic hybridization: a strategy to reduce reference channel hybridizations.* Genes Chromosomes Cancer, 2008. **47**(11): p. 994-1004.

38. Edelmann, L. and K. Hirschhorn, *Clinical utility of array CGH for the detection of chromosomal imbalances associated with mental retardation and multiple congenital anomalies.* Ann N Y Acad Sci, 2009. **1151**: p. 157-66.

39. Li, D. and R. Roberts, *WD-repeat proteins: structure characteristics, biological function, and their involvement in human diseases.* Cell Mol Life Sci, 2001. **58**(14): p. 2085-97.

40. Fritzius, T., et al., *A WD-FYVE protein binds to the kinases Akt and PKCzeta/lambda.* Biochem J, 2006. **399**(1): p. 9-20.

41. Lim, J., et al., *A protein-protein interaction network for human inherited ataxias and disorders of Purkinje cell degeneration.* Cell, 2006. **125**(4): p. 801-14.

42. Neve, E.P., et al., *VIPL, a VIP36-like membrane protein with a putative function in the export of glycoproteins from the endoplasmic reticulum.* Exp Cell Res, 2003. **288**(1): p. 70-83.

43. Tran, T.S., A.L. Kolodkin, and R. Bharadwaj, *Semaphorin regulation of cellular morphology.* Annu Rev Cell Dev Biol, 2007. **23**: p. 263-92.

44. Colon-Ramos, D.A., M.A. Margeta, and K. Shen, *Glia promote local synaptogenesis through UNC-6 (netrin) signaling in C. elegans.* Science, 2007. **318**(5847): p. 103-6.

45. Maier, V., et al., *Semaphorin 4C and 4G are ligands of Plexin-B2 required in cerebellar development.* Mol Cell Neurosci, 2011. **46**(2): p. 419-31.

46. Qin, Y., et al., *Germline mutations in TMEM127 confer susceptibility to pheochromocytoma.* Nat Genet, 2010. **42**(3): p. 229-33.

47. Rudd, M.K., et al., *Segmental duplications mediate novel, clinically relevant chromosome rearrangements.* Hum Mol Genet, 2009. **18**(16): p. 2957-62.

48. Imai, Y., et al., *An unfolded putative transmembrane polypeptide, which can lead to endoplasmic reticulum stress, is a substrate of Parkin.* Cell, 2001. **105**(7): p. 891-902.

49. Rossini, L., et al., *VSTM2L is a novel secreted antagonist of the neuroprotective peptide Humanin.* FASEB J, 2011. **25**(6): p. 1983-2000.

50. Chantret, I., et al., *Congenital disorders of glycosylation type Ig is defined by a deficiency in dolichyl-P-mannose:Man7GlcNAc2-PP-dolichyl mannosyltransferase.* J Biol Chem, 2002. **277**(28): p. 25815-22.

51. Grubenmann, C.E., et al., *ALG12 mannosyltransferase defect in congenital disorder of glycosylation type lg.* Hum Mol Genet, 2002. **11**(19): p. 2331-9.

52. Froyen, G., et al., *Submicroscopic duplications of the hydroxysteroid dehydrogenase HSD17B10 and the E3 ubiquitin ligase HUWE1 are associated with mental retardation.* Am J Hum Genet, 2008. **82**(2): p. 432-43.

53. Tarpey, P.S., et al., *Mutations in CUL4B, which encodes a ubiquitin E3 ligase subunit, cause an X-linked mental retardation syndrome associated with aggressive outbursts, seizures, relative macrocephaly, central obesity, hypogonadism, pes cavus, and tremor.* Am J Hum Genet, 2007. **80**(2): p. 345-52.

54. Nascimento, R.M., et al., *UBE2A, which encodes a ubiquitin-conjugating enzyme, is mutated in a novel X-linked mental retardation syndrome.* Am J Hum Genet, 2006. **79**(3): p. 549-55.

55. Kishino, T., M. Lalande, and J. Wagstaff, *UBE3A/E6-AP mutations cause Angelman syndrome.* Nat Genet, 1997. **15**(1): p. 70-3.

56. Ross, C.A. and C.M. Pickart, *The ubiquitin-proteasome pathway in Parkinson's disease and other neurodegenerative diseases.* Trends Cell Biol, 2004. **14**(12): p. 703-11.

57. Bennett, E.J., et al., *Global changes to the ubiquitin system in Huntington's disease.* Nature, 2007. **448**(7154): p. 704-8.

58. Kenwrick, S., A. Watkins, and E. De Angelis, *Neural cell recognition molecule L1: relating biological complexity to human disease mutations.* Hum Mol Genet, 2000. **9**(6): p. 879-86.

59. Traut, W., et al., *New members of the neurexin superfamily: multiple rodent homologues of the human CASPR5 gene.* Mamm Genome, 2006. **17**(7): p. 723-31.

60. Sudhof, T.C., *Neuroligins and neurexins link synaptic function to cognitive disease.* Nature, 2008. **455**(7215): p. 903-11.

61. Edwards, Y.H., et al., *A novel human phosphoglucomutase (PGM5) maps to the centromeric region of chromosome 9.* Genomics, 1995. **30**(2): p. 350-3.

62. Li, J., et al., *Association between tryptophan hydroxylase gene polymorphisms and attention deficit hyperactivity disorder in Chinese Han population.* Am J Med Genet B Neuropsychiatr Genet, 2006. **141B**(2): p. 126-9.

63. Bellivier, F., P. Chaste, and A. Malafosse, *Association between the TPH gene A218C polymorphism and suicidal behavior: a meta-analysis.* Am J Med Genet B Neuropsychiatr Genet, 2004. **124B**(1): p. 87-91.

64. Stefulj, J., et al., *TPH gene polymorphism and aging: indication of combined effect on the predisposition to violent suicide.* Am J Med Genet B Neuropsychiatr Genet, 2006. **141B**(2): p. 139-41.

65. Allen, N.C., et al., *Systematic meta-analyses and field synopsis of genetic association studies in schizophrenia: the SzGene database.* Nat Genet, 2008. **40**(7): p. 827-34.

66. Espinosa, F., et al., *Ablation of Kv3.1 and Kv3.3 potassium channels disrupts thalamocortical oscillations in vitro and in vivo.* J Neurosci, 2008. **28**(21): p. 5570-81.

67. Espinosa, F., et al., *Increased motor drive and sleep loss in mice lacking Kv3-type potassium channels.* Genes Brain Behav, 2004. **3**(2): p. 90-100.

68. Steinlein, O.K., et al., *An insertion mutation of the CHRNA4 gene in a family with autosomal dominant nocturnal frontal lobe epilepsy.* Hum Mol Genet, 1997. **6**(6): p. 943-7.

69. Biervert, C., et al., *A potassium channel mutation in neonatal human epilepsy.* Science, 1998. **279**(5349): p. 403-6.

70. Singh, N.A., et al., *A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns.* Nat Genet, 1998. **18**(1): p. 25-9.

71. Cooper, E.C. and L.Y. Jan, *M-channels: neurological diseases, neuromodulation, and drug development.* Arch Neurol, 2003. **60**(4): p. 496-500.

72. Zhou, X., et al., *Novel role of KCNQ2/3 channels in regulating neuronal cell viability.* Cell Death Differ, 2011. **18**(3): p. 493-505.

73. Jongbloed, R., et al., *DHPLC analysis of potassium ion channel genes in congenital long QT syndrome.* Hum Mutat, 2002. **20**(5): p. 382-91.

74. Hofman, N., et al., *Recurrent and Founder Mutations in the Netherlands: the Long-QT Syndrome.* Neth Heart J, 2011. **19**(1): p. 10-16.

75. Zahir, F. and J.M. Friedman, *The impact of array genomic hybridization on mental retardation research: a review of current technologies and their clinical utility.* Clin Genet, 2007. **72**(4): p. 271-87.

76. Daniela, F. and T. Lopes, *Genomic imbalances in patients with intellectual disability*, in *Chemistry Department*. 2011, Aveiro university: Aveiro

77. Curry, C.J., et al., *Homozygous deletions of a copy number change detected by array CGH: a new cause for mental retardation?* Am J Med Genet A, 2008. **146A**(15): p. 1903-10.

78. Lee, C., A.J. Iafrate, and A.R. Brothman, *Copy number variations and clinical cytogenetic diagnosis of constitutional disorders.* Nat Genet, 2007. **39**(7 Suppl): p. S48-54.

79. Chen, J., *Molecular Basis of Gene Dosage Sensitivity*. 2009, Rice University: Houston, Texas.

80. Bisgaard, A.M., et al., *Transmitted cytogenetic abnormalities in patients with mental retardation: pathogenic or normal variants?* Eur J Med Genet, 2007. **50**(4): p. 243-55.

81. Tsuang, M.T., et al., *Gene-environment interactions in mental disorders.* World Psychiatry, 2004. **3**(2): p. 73-83.

82. Kronenberg, M.E., S. Raz, and C.J. Sander, *Neurodevelopmental outcome in children born to mothers with hypertension in pregnancy: the significance of suboptimal intrauterine growth.* Dev Med Child Neurol, 2006. **48**(3): p. 200-6.

83. van Schie, P.E., et al., *General movements in infants born from mothers with early-onset hypertensive disorders of pregnancy in relation to one year's neurodevelopmental outcome.* Early Hum Dev, 2008. **84**(9): p. 605-11.

84. Leblond, C.S., et al., *Genetic and Functional Analyses of SHANK2 Mutations Suggest a Multiple Hit Model of Autism Spectrum Disorders.* PLoS Genet, 2012. **8**(2): p. e1002521.

85. Shaffer, L.G. and B.A. Bejjani, *Medical applications of array CGH and the transformation of clinical cytogenetics.* Cytogenet Genome Res, 2006. **115**(3-4): p. 303-9.

APPENDIX

APPENDIX 1.

CONSENT FORM

Consentimento informado para participação no estudo de investigação:

"Estudo genético do défice cognitivo"

Considerando a "Declaração de Helsinquia" da Associação Médica Mundial (1964; Tóquio 1975; Veneza 1983; Hong Kong 1989; Somerset West 1996 e Edimburgo 2000)

Estudo

Designação (em português):_____

_____

Investigador responsável:_____

Categoria profissional:_____

Instituição:_____

Processo clínico nº: _____ * riscar o que não interessa

Eu, abaixo-assinado, (nome completo) _____

_____, portador do BI nº _____,

responsável pelo participante no estudo (nome completo) _____

_____,compreendi a explicação que me foi fornecida acerca da minha/sua* situação clínica e da investigação que se tenciona realizar, bem como do estudo em que serei/será* incluído. Tive a oportunidade de fazer todas as perguntas que julguei necessárias tendo obtido resposta satisfatória.

Compreendi os objectivos, métodos, benefícios previstos e riscos potenciais do estudo. Autorizo que a informação e dados iconográficos respeitantes a mim/ele(a)* sejam partilhados, de forma anónima, com a comunidade científica.

Além disso, foi-me afirmado que tenho o direito de recusar em qualquer momento a participação no estudo, sem necessidade de dar qualquer explicação e sem que isso possa ter como efeito qualquer prejuízo na assistência que me/lhe* é prestada, sendo assegurada a confidencialidade da identidade e registos associados.

AUTORIZAÇÃO DO PRÓPRIO / REPRESENTANTE LEGAL

Autorizo a minha/sua* inclusão no referido estudo.

Assinatura: _____ Data: ___ de _____ de _____

A PREENCHER PELO MÉDICO

Confirmo ter informado, de forma adequada e compreensível, sobre os objectivos, métodos, benefícios previstos e riscos potenciais do estudo

Nome do Médico (legível):_____ Céd.Prof :_____

Assinatura:_____ Instituição:_____

ANULAÇÃO DO CONSENTIMENTO INFORMADO Data: ___/___/___

Eu, _____ **anulo** o consentimento dado na data de ___/___/_____. **Não autorizo que** _____ **continue a participar neste estudo**, pelo que nesta data o dou por finalizado, estando consciente desta decisão.

(O Próprio / representante legal) (O Médico) (O Investigador responsável)

Para mais informações sobre o projecto ou sobre como participar contacte:

Instituto de Ciências da Vida e da Saúde – Prof. Dr.ª Patrícia Maciel (253604824);

Hospital Pediátrico de Coimbra - Prof. Dr.ª Guiomar Oliveira, Dr Frederico Duque, Dra Carla Marques (239480625/626/621).

Consentimento informado para participação no estudo de investigação:
"Estudo genético do défice cognitivo"

Considerando a "Declaração de Helsinquia" da Associação Médica Mundial
(1964; Tóquio 1975; Veneza 1983; Hong Kong 1989; Somerset West 1996 e Edimburgo 2000)

O presente estudo foca-se em crianças com défice cognitivo sem etiologia (causa) conhecida, com ou sem evidência familiar de ligação ao cromossoma X. O objectivo da vossa participação neste estudo é o de pesquisar a causa do défice cognitivo no material genético obtido através de colheita de sangue. Na maior parte dos casos a análise terá como base um painel de genes, quase todos localizados no cromossoma X para procurar mutações causadoras desta patologia do neurodesenvolvimento. Alguns indivíduos do estudo serão igualmente submetidos a outra análise genética usando uma tecnologia denominada *Array comparative genomic hybridization* (aCGH), que consiste numa pesquisa global do genoma permitindo a identificação de perdas ou duplicações de pequenas regiões de todos os cromossomas.

As crianças que participam no estudo serão observadas por uma equipa multidisciplinar de que faz parte um pediatra do neurodesenvolvimento, um médico geneticista (quando necessário), e um psicólogo.

As amostras de sangue das crianças, dos pais (e/ou, sempre que possível ou necessário, de outros familiares) serão utilizadas para realizar a extracção de ADN e efectuar a/s análise/s genética/s. Após a colheita, o sangue será enviado para o *Instituto de Ciências da Vida e Saúde* (ICVS) da Universidade do Minho, em Braga, onde decorre a pesquisa de mutações, garantindo-se o **anonimato** no manuseamento das amostras e dos dados clínicos recolhidos.

Cumprindo todos os esforços para garantir a protecção dos dados dos participantes, e de acordo com as obrigações legais e éticas em vigor, as amostras são codificadas, pelo que o nome de cada individuo é codificado e os dados pessoais permanecem confidenciais, sendo o seu acesso e cruzamento com informação clínica e molecular muito limitado. Foi ainda criada uma base de dados online, com acesso igualmente restrito à equipa deste projecto, para partilha de informações exclusivamente anónimas e não passíveis de identificação. As amostras biológicas, bem como os dados clínicos recolhidos, irão permanecer armazenados e codificados em banco, no ICVS, por período indefinido. No caso de necessitar da amostra biológica para outros fins, por favor entre em contacto com os responsáveis pelo estudo.

Até ao final de 2011, será enviado um relatório a cada família participante, assinalando se foram encontradas ou não mutações ou alterações genómicas que expliquem a causa do défice cognitivo do seu/sua filho/a. Durante este período, se tiver alguma dúvida deverá contactar o médico responsável pelo estudo do seu/sua filho/a que lhe facultará a informação disponível.

É-lhe dado(a) desde o início a opção de recusar ser contactado(a) novamente, sem fornecer motivos, pelo que deve expressar essa vontade por escrito. Tem também a possibilidade de desistir do estudo em qualquer momento, sem fornecer motivos ou sofrer qualquer penalização, bastando indicar por escrito esse desejo. Deverá nesse caso, indicar ainda se a amostra e os dados recolhidos até então poderão continuar a ser utilizados de forma anónima, ou se pretende a exclusão completa incluindo a destruição de todos os dados e amostras.

No global, este estudo deve contribuir para a identificação de alterações genéticas responsáveis por 10 a 20% dos casos de défice cognitivo inicialmente de causa desconhecida, permitindo assim demonstrar a utilidade da análise dum painel de genes deste tipo, bem como da tecnologia aCGH, no diagnóstico etiológico do défice cognitivo em contexto clínico.

A identificação da causa genética do défice cognitivo do seu/sua filho(a) tem implicações relevantes, desde a conclusão do processo de diagnóstico, ao estabelecimento do prognóstico, à adequação de medidas terapêuticas e educacionais, à participação em associações, bem como à possibilidade de melhorar o aconselhamento genético da família quanto ao risco de recorrência.

APPENDIX 2.

SUPLEMENTARY TABLES

Table S1-S6 (patient 1-6, respectively). Alterations found by array-CGH in patient 1-6, respectively (**Green underlined**: unknown clinical significance/**Red underlined:** alterations that required further study/**No underline**: alterations found in more than 3 controls in databases).

Chromosome Region	Event	Cytoband
chr1:12,756,666-13,013,869	CN Gain	p36.21
chr1:16,725,089-17,077,550	CN Gain	p36.13
chr2:242,507,916-242,671,783	CN Loss	q37.3
chr3:75,532,046-75,784,380	CN Gain	p12.3
chr3:163,987,281-164,108,596	CN Gain	q26.1
chr4:68,954,035-69,207,266	CN Loss	q13.2
chr5:736,094-915,050	CN Gain	p15.33
chr5:68,833,867-70,635,856	CN Gain	q13.2
chr6:193,938-286,758	CN Loss	p25.3
chr8:39,349,059-39,508,365	Homozygous Copy Loss	p11.23 - p11.22
chr10:45,969,999-46,703,566	CN Gain	q11.21 - q11.22
chr13:51,009,338-51,167,405	CN Gain	q14.3
chr15:18,362,555-18,586,588	CN Loss	q11.1 - q11.2
chr15:18,586,588-20,154,336	CN Gain	q11.2
chr16:28,786,465-28,984,444	CN Gain	p11.2
chr17:41,464,040-41,713,328	CN Gain	q21.31
chr19:48,196,695-48,496,066	CN Loss	q13.31
chr22:23,983,829-24,294,003	High Copy Gain	q11.23

Chromosome Region	Event	Cytoband
chr1:16,746,886-16,962,625	CN Gain	p36.13
chr1:16,962,625-17,064,137	CN Loss	p36.13
chr1:17,064,137-17,148,158	CN Gain	p36.13
chr1:72,532,314-72,590,954	Homozygous Copy Loss	p31.1
chr2:241,261,124-241,375,841	CN Gain	q37.3
chr3:163,987,281-164,108,596	High Copy Gain	q26.1
chr3:196,848,435-196,956,726	CN Gain	q29
chr4:69,008,654-69,165,944	CN Gain	q13.2
chr5:745,872-900,209	CN Gain	p15.33
chr5:68,815,697-70,696,119	CN Loss	q13.2
chr7:143,548,913-143,667,377	CN Loss	q35
chr8:7,214,279-7,942,536	CN Loss	p23.1
chr11:31,508,016-31,797,706	CN Loss	p13
chr14:105,637,917-105,854,362	CN Gain	q32.33
chr15:32,469,479-32,674,648	CN Loss	q14

Chromosome Region	Event	Cytoband
chr1:102,431,081-102,633,897	CN Loss	p21.1
chr1:142,117,366-142,453,717	CN Loss	q12 - q21.1
chr1:194,993,686-195,097,982	CN Gain	q31.3
chr2:91,273,082-91,625,698	CN Loss	p11.1
chr2:96,098,910-97,594,697	CN Gain	q11.2
chr2:130,418,126-130,467,710	CN Loss	q21.1
chr2:131,811,198-131,860,657	CN Loss	q21.1
chr3:75,752,964-75,905,213	CN Loss	p12.3
chr3:163,987,281-164,108,596	High Copy Gain	q26.1
chr4:34,448,772-34,500,505	CN Gain	p15.1
chr4:68,978,187-69,170,341	CN Gain	q13.2
chr4:70,227,242-70,269,497	CN Loss	q13.2

chr5:742,057-839,349	CN Gain	p15.33
chr5:70,245,600-70,375,604	CN Gain	q13.2
chr6:193,938-327,656	CN Loss	p25.3
chr6:32,548,396-32,600,341	Homozygous Copy Loss	p21.32
chr6:32,600,341-32,623,614	CN Gain	p21.32
chr6:32,623,614-32,681,379	CN Loss	p21.32
chr6:79,027,678-79,098,580	CN Gain	q14.1
chr6:168,076,174-168,333,368	CN Gain	q27
chr7:38,284,089-38,349,754	CN Loss	p14.1
chr7:124,186,384-124,227,026	High Copy Gain	q31.33
chr7:142,066,473-142,209,840	CN Loss	q34
chr7:142,947,514-143,049,236	CN Gain	q35
chr8:12,270,256-12,366,919	Homozygous Copy Loss	p23.1
chr8:12,366,919-12,554,253	CN Loss	p23.1
chr8:39,349,059-39,508,365	High Copy Gain	p11.23 - p11.22
chr9:39,999,379-40,265,103	CN Loss	p13.1 - p12
chr9:65,525,874-65,792,243	CN Loss	q12
chr10:38,948,353-39,080,121	CN Loss	p11.1
chr10:45,969,999-46,573,606	CN Loss	q11.21 - q11.22
chr10:47,169,331-47,451,593	CN Loss	q11.22
chr10:48,381,494-49,040,567	CN Loss	q11.22
chr11:18,851,036-18,925,523	CN Loss	p15.1
chr11:55,119,157-55,178,810	CN Gain	q11
chr12:8,208,895-8,273,494	CN Loss	p13.31
chr12:31,170,795-31,292,064	CN Gain	p11.21
chr14:18,436,931-19,490,518	CN Loss	q11.1 - q11.2
chr14:40,669,880-40,734,612	CN Loss	q21.1
chr14:105,602,384-105,693,838	CN Loss	q32.33
chr15:18,561,627-20,154,336	CN Loss	q11.2
chr16:22,377,707-22,631,014	CN Loss	p12.1
chr16:32,096,628-32,582,495	CN Loss	p11.2
chr16:33,230,071-33,720,310	CN Loss	p11.2
chr16:34,345,125-34,600,842	CN Loss	p11.2 - p11.1
chr16:58,848,869-59,019,408	CN Loss	q21
chr16:68,697,986-68,774,654	CN Gain	q22.1
chr17:31,459,116-31,511,535	CN Loss	q12
chr17:41,577,131-41,768,841	CN Gain	q21.31
chr19:17,330,586-17,377,389	CN Gain	p13.11
chr19:20,067,301-20,148,413	CN Loss	p12
chr19:20,374,751-20,502,094	CN Loss	p12
chr22:14,433,473-14,629,187	CN Loss	q11.1
chr22:17,036,669-17,240,532	CN Loss	q11.21

Chromosome Region	**Event**	**Cytoband**
chr4:4,060,480-4,243,929	CN Loss	p16.2
chr4:190,715,999-190,919,040	CN Loss	q35.2
chr5:53,496,401-53,681,084	CN Gain	q11.2
chr6:193,938-327,656	CN Loss	p25.3
chr7:76,078,704-76,408,872	CN Loss	q11.23
chr8:39,349,059-39,508,365	High Copy Gain	p11.23 - p11.22
chr10:81,053,595-81,667,828	CN Loss	q22.3
chr11:4,207,201-4,333,826	Homozygous Copy Loss	p15.4
chr14:18,475,668-19,490,518	CN Gain	q11.1 - q11.2
chr15:19,033,127-20,258,362	CN Loss	q11.2
chr16:32,437,818-33,691,406	CN Loss	p11.2
chr17:78,632,251-78,774,742	CN Loss	q25.3
chr18:27,570,289-27,823,851	CN Loss	q12.1
chr20:35,964,534-36,052,172	CN Gain	q11.23

Chromosome Region	**Event**	**Cytoband**
chr1:194,993,686-195,097,982	CN Gain	q31.3
chr3:163,987,281-164,108,596	High Copy Gain	q26.1

chr4:69,008,654-69,170,341	CN Loss	q13.2
chr5:68,938,168-70,043,543	CN Gain	q13.2
chr6:79,027,678-79,098,580	CN Loss	q14.1
chr7:143,549,135-143,693,789	CN Loss	q35
chr7:158,787,126-158,821,424	CN Loss	q36.3
chr8:7,214,279-7,942,536	CN Loss	p23.1
chr10:27,645,920-27,751,746	CN Loss	p12.1
chr10:96,612,594-97,307,249	CN Loss	q23.33
chr14:105,136,197-105,257,495	CN Gain	q32.33
chr15:18,643,606-20,154,336	CN Loss	q11.2
chr17:41,521,663-41,703,816	High Copy Gain	q21.31
chr17:78,638,125-78,774,742	CN Gain	q25.3
chr18:0-131,060	CN Gain	p11.32
chr20:62,400,822-62,435,964	CN Loss	q13.33
chr22:48,647,628-48,781,558	CN Gain	q13.33
chrX:53,586,378-53,786,473	High Copy Gain	p11.22
chrX:103,138,374-103,207,921	High Copy Gain	q22.2
chrX:152,783,739-152,935,572	CN Gain	q28

Chromosome Region	**Event**	**Cytoband**
chr1:72,532,314-72,590,954	Homozygous Copy Loss	p31.1
chr1:147,255,457-147,449,377	Homozygous Copy Loss	q21.1
chr1:147,449,377-147,753,238	CN Loss	q21.1
chr1:194,993,686-195,078,812	CN Loss	q31.3
chr2:94,730,661-94,887,128	CN Gain	q11.1
chr3:101,814,939-101,924,845	CN Gain	q12.2
chr3:163,987,281-164,108,596	Homozygous Copy Loss	q26.1
chr4:34,448,772-34,500,505	CN Gain	p15.1
chr4:48,953,993-49,276,565	CN Gain	p11
chr4:70,170,112-70,285,238	CN Gain	q13.2
chr5:726,908-933,798	CN Loss	p15.33
chr5:68,877,031-70,671,221	CN Gain	q13.2
chr6:32,548,396-32,666,536	High Copy Gain	p21.32
chr6:79,027,678-79,098,580	CN Gain	q14.1
chr9:38,942,543-46,984,229	CN Gain	p13.1 - p11.1
chr9:46,984,229-47,055,569	High Copy Gain	p11.1
chr9:65,426,303-70,197,877	CN Gain	q12 - q13
chr11:17,642,822-18,671,307	CN Gain	p15.1
chr11:89,247,728-89,320,683	CN Gain	q14.3
chr11:89,320,683-89,430,796	CN Loss	q14.3
chr11:89,430,796-89,556,616	High Copy Gain	q14.3
chr12:9,519,693-9,606,536	Homozygous Copy Loss	p13.31
chr14:18,794,098-19,490,518	CN Gain	q11.1 - q11.2
chr14:20,418,144-20,485,700	CN Loss	q11.2
chr14:105,136,197-105,283,501	CN Loss	q32.33
chr15:19,441,979-19,796,307	CN Gain	q11.2
chr15:19,796,307-20,154,336	CN Loss	q11.2
chr16:33,285,183-33,662,422	CN Gain	p11.2
chr16:54,345,532-54,383,408	Homozygous Copy Loss	q12.2
chr17:33,338,413-33,649,919	CN Loss	q12
chr17:41,521,663-41,768,841	CN Loss	q21.31
chr17:41,768,841-42,128,214	CN Gain	q21.31 - q21.32
chr19:428,087-475,533	CN Gain	p13.3
chr19:20,374,751-20,502,094	CN Loss	p12
chr19:48,012,670-48,252,042	CN Loss	q13.31
chr20:27,100,000-28,266,113	CN Gain	q11.1
chr20:61,268,624-61,790,781	CN Gain	q13.33
chr22:17,036,669-17,240,532	CN Loss	q11.21

Tabela S7. Summary of some of the alterations that were not considered benign variants and required further investigations to assess their clinical significance taking into account the type of event, size, gene content and inheritance.

Patient nº	Nº of alterations found	Event	Chromosome Region	Size (Mb)	Nº of genes	Genes in the region (not all listed)	Candidate genes for ID	Inheritance
1	17	Dup (13) (13q14.3)	chr13:51,009,338-51,167,405	0,24	3	MIR4703, WDF2, WDFY2	WDFY2	Father
2	15	-	-	-	-	-	-	-
3	54	Dup (2) (2q11.2)	chr2:96,098,910-97,594,697	1,5	24	ADRA2B, ANKRD23, ANKRD36, ANKRD36B, ANKRD39, ARID5A, ASTL, CIAO1, CNNM3, CNNM4, DUSP2, FAHD2B, FAM178B, FER1L5, ITPRIPL1, KIAA1310, LMAN2L, LOC285033, NCAPH, NEURL3, SEMA4C, SNRNP200, STARD7, TMEM127	**ADRA2B**, ARID5A, **CNNM3**, ITPRIPL1, LMAN2L, **SEMA4C**, **TMEM127**	Mother
		Dup (7) (7q31.33)	chr7:124,186,384-124,227,026	0,04	1	GPR37	GPR37*	Unknown
4	14	Dup (5) (5q11.2)	chr5:53,496,401-53,681,084	0,18	1	ARL15	-	Father
		Del (18) (18q12.1)	chr18:27,570,289-27,823,851	0,25	2	KIAA1012, MCART2	KIAA1012	Father
		Dup (20) (20q11.23)	chr20:35,964,534-36,052,172	0,98	2	KIAA0406, VSTM2L	VSTM2L	*De novo*
5	20	Del (10) (10q23.3)	chr10:96,612,594-97,307,249	0,7	5	C10orf129, CYP2C8, CYP2C9, PDLIM1, SORBS1	-	Father
		Dup (22) (22q13.33)	chr22:48,647,628-48,781,558	0,13	5	ALG12, CRELD2, IL17REL, PIM3, ZBED4	ALG12	*De novo*
		Dup (X) (Xp11.22)	chrX:53,586,378-53,786,473	0,2	3	HUWE1, MIR98, MIRLET7F2	HUWE1*	Mother

		Dup (X) (Xq28)	chrX:152,783,739-152,935,572	0,15	8	ARHGAP4, AVPR2, HCFC1, IRAK1, L1CAM, NAA10, RENBP, TMEM187	L1CAM	*De novo*
6	38	Dup (9) (p13.1-p11.1)	chr9:38,942,543-46,984,229	8	58	CBWD5, CCDC29, CNTNAP3, CNTNAP3B, CR605783, CR615666, DKFZp572C163, DKFZp686I15204, FOXD4L4, KGFLP1, KGFLP2, KIAA171, MGC21881, ZNF658	CNTNAP3, ZNF68	Mother
		Dup (9) (q12-q13)	chr9:65,426,303-70,197,877	4,8	49	CBWD3, CBWD5, CBWD6, CCDC29, CR615453, CR626459, CR627148, DKFZp434A171, FAM27E3, FOXD4L2, FOXD4L3, FOXD4L5, FOXD4L6, MGC21881, PGM5, PGM5P2	-	Mother
		Dup (11)(p15.1)	chr11:17,642,822-18,671,307	1	27	DelGEF, GTF2H1, HPS5, KCNC1, KIAA1017, LDHA, LDHAL6A, LDHC, MRGPRX3, MRGPRX4, MYOD1, SAA1, SAA2, SAA3, SAA4, , SERGEF, SPTY2D1, TPH1, TSG101, UEVLD	DelGEF, HPS5, KCNC1,TPH1	Father
		Dup (20) (q13.33)	chr20:61,268,624-61,790,781	0,5	29	AK056267, ARFGAP1, AY940852, BIRC7, C20orf149, C20orf195, CHRNA4, COL20A1, EEF1A2, FLJ16779, GMEB2, HRIHFB2281, KCNQ2, KIAA1269, KIAA1510, MIR124-3, MIR3196, MIR4326, NKAIN4, PPDPF, PRIC285, PTK6, RP4-697K14.11, RTEL1, RTEL1-TNFRSF6B, SCLIP, SRMS, STMN3, YTHDF1, hsa-mir-124-3	ARFGAP1, CHRNA4, EEF1A2, GMEB2, KCNQ2, NKAIN4, SCLIP	Mother