

Research Article

Genetic Analysis in the Diagnosis of Intellectual Disabilities in Adults

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Abstract

Background: High-resolution technologies such as array Comparative Genomic Hybridization (aCGH) are changing the diagnostic approach to complex disorders as Intellectual Disability (ID) in such manner, that it is being advocated to be the first-tier genetic testing for the diagnostic protocol in ID patients. According to this premise, we conducted a study in a cohort of institutionalized adult ID patients.

Method: 121 institutionalized idiopathic ID adult patients were tested by analytical procedures as standard karyotype, metabolic analysis, DNA testing for Fragile X and aCGH array. aCGH array was carried out from genomic DNA purified from blood samples by applying the Neuroarray technology (Genetadi, Bilbao, Spain).

Results: We identified a genetic cause in 80% of our sample where 71% of them have cryptic chromosomal abnormalities, 4% Fragile X syndrome (FRAXA) and 5% Inborn Errors of Metabolism (IEM). There is a predominance of microdeletions over microduplications being the 17q microdeletion the most frequent cryptic chromosomal abnormality detected in our sample.

Conclusion: According to this study we suggest that applying the aCGH technology as a first step within a diagnostic algorithm we will be able to increase the diagnostic accuracy reducing the rate of idiopathic forms.

Keywords: Cryptic Chromosomal Abnormality; Fragile X; Inborn Errors of Metabolism; Intellectual Disability; Mental Retardation; Microdeletion; Microduplication; aCGH Array; 17q Microdeletion

Introduction

Intellectual Disability (ID, previously known as known as mental retardation) is a condition included in the neurodevelopment disorder group which is characterized by significant limitations in both intellectual functioning and in adaptive behavior covering many everyday social and practical skills having an onset prior to 18 years of age [1]. The reported prevalence of ID ranges between 1 and 3% being higher among male versus female [2]. It depends on many variables as population (age, gender, social class and ethnicity) and criteria and methods of assessment used [2,3]. The cause of most ID patients is unknown. In fact, there are a het-

erogeneous group of factors associated with increased risk of ID. Basically, these factors are classified as genetic and non-genetic factors with different contribution depending on each particular context and the moment of impact [4]. The most frequent genetic conditions are represented by chromosomal abnormalities followed by single-gene defects where inborn errors of metabolism are also included [5-7]. Among the non-genetic factors, we have to consider the moment of their impact: being of prenatal, perinatal or postnatal influence. Prenatal non-genetics factors are mainly represented by viral infections (e.g. toxoplasmosis, rubella) [8], drugs and toxic influences (e.g. alcohol) [9], dietary factors as iodine deficiency [10] and Rh incompatibility [11]. Among perinatal non-genetics factors the most outstanding are prematurity and a traumatic labor delivery which induces anoxia and brain damage [12], whereas among the most relevant postnatal influences are infections [13,14], poisonings, cerebral trauma (head injury) [15] or

sociocultural factors [16].

In spite of that fact, in any case and independently of the stage we will always be in front of the dilemma to discern the real contribution of genetic and non-genetic variables in each patient in order to ensure the best genetic counseling for the family [17]. In general, it is estimated a recurrence risk of 3-9% for families with one previous child with severe intellectual disability [18]. However, this risk has to be adjusted according to each specific condition. At this point, genetic analysis is an indispensable tool to clarify the causes related to family risks.

Genetic Approach

When considering genetic factors as cause of ID, it is important to recognize that approximately 10-40% of patients have a detectable chromosome abnormality [19]. Most of them show a recognizable pattern of clinical characteristics (distinguishing phenotype) which makes possible to suppose the diagnosis (e.g. Down syndrome or trisomy 21). However, many cases may not be so obvious to be clinically recognizable and we have to resort to algorithms designed to facilitate the diagnostic process. Taking into account those considerations, after an exhaustive clinical evaluation, the first test to be indicated is the chromosomal analysis (Karyotyping). Other patients, some of them with an atypical phenotype, may have small chromosomal rearrangements (deletions or duplications) non-detectable by karyotype which is contributing to an upward revision of chromosomal involvement in ID etiology [20].

Nevertheless, conventional chromosome analysis or karyotype has a limited resolution since it is only able to detect defects larger than 3 Mb [21]. There are some other techniques to look for smaller chromosomal abnormalities as Multiplex Ligation-Dependent Probe Amplification (MLPA) which is used to test sub telomeric regions of all chromosomes or like Fluorescent in Situ Hybridization (FISH) in order to confirm a clinical diagnosis of some well-known microdeletion/microduplication syndromes (e.g. Williams-Beuren syndrome, DiGeorge syndrome). Both time-consuming techniques are also carried out in specialized laboratories which combine conventional and molecular cytogenetics [22,23].

The chromosome microarray or comparative genomic hybridization technique (array-CGH) is the most recent technique to be used in genetic study of ID. It has the advantage to incorporate in a unique test the ability to identify microdeletions and/or microduplications with the highest degree of sensitivity (25-50 kb) [21]. In this sense, array-CGH is pointing out that it is necessary an upward recalculation of the frequency of chromosomal abnormalities in ID [24,25]. By definition a Copy Number Variations (CNVs) is an increased or decreased number of copies of a DNA fragment causing a deviation from the normal diploid pattern. There are CNVs which represent benign variations whereas others are responsible of a clinical phenotype [26]. Most of detected CNVs are

de novo but some of them may be inherited from a parent. This is an important point to clarify to assure a correct genetic counseling to the family [27].

In spite of chromosomal causes, there are also monogenic causes explaining some of the inherited forms of ID [28]. The most common inherited form of monogenic ID, particularly in males, is the Fragile X syndrome [29,30]. Its prevalence is 1 in 3,600 in males and approximately 1 in 4,000 to 6,000 in females. The defective gene is the FMR1 which is located in the long arm of chromosome X (Xq27.3) [31]. Most cases, especially adults, are easily identifiable by clinical examination because their peculiar phenotype (Martin-Bell phenotype), nevertheless, it is important to confirm the diagnosis by mean of the DNA testing to evaluate the FMR1 status following the guidelines defined by the American College of Medical Genetics (ACMG) [32].

There are many other X-linked and autosomal monogenic causes of ID. In chromosome X are described more than 80 gene defects associated with ID [33] and in monogenic chromosomes there are described more than 1400 genes and the list is still increasing for both syndromic and non-syndromic forms due to the latest technological improvements [28]. Metabolic disorders, which are also monogenic in origin, are another important cause of intellectual disabilities to be considered in diagnosis protocol applied to idiopathic ID [34]. Maybe its relevance was more pronounced in the past because today neonatal screenings are reducing their impact on this condition [35].

Subjects and Methods

Subjects

In the present study were included 121 adults (77 men, 44 women; age range 20-70 yrs.) with intellectual disabilities and dysmorphic pattern (in all of them are present abnormalities in cranium and face as well as hands and feet). They are all residing in an institution for the mentally retarded people ("Group Catalonia", Barcelona, Spain) with an average time of institutionalization of 19,7 years.

Data Collection

Clinical records and institutional archives were searched to collect data from each patient. Data were selected from diagnosis collected during 2013 with the objective to know possible etiologies of those patients who were previously classified as unknown etiologies. All patients were examined by one physician with expertise in clinical genetics and by clinical psychologists to delimit the clinical phenotype to be included in the study.

Inclusion Criteria

Three groups of patients were included according to the following characteristics:

- Patients with moderate-severe intellectual disabilities and dysmorphic features of unknown etiology.
- Patients with moderate-severe intellectual disabilities and genetic diagnosis.
- Undiagnosed patients with clinical features suggesting a recognizable intellectual disabilities syndrome.

Laboratory Investigations

Chromosome Analysis

Chromosome preparations from peripheral blood cultures and cytogenetic analyses were performed using a high-resolution karyotype (850 bands).

FMR1 Molecular Analysis

The presence of an FMR1 gene expansion was analysed using molecular PCR procedures followed by fragment analysis by capillary electrophoresis (AmplideXtm FMR1PCRKit).

Metabolic Studies

An analytical study on dried blood of metabolic markers (analytes) was performed using an expanded neonatal screening test based on the tandem mass spectrometry (MS/MS) technology (NEONATAL-ONE®, Genetadi) which covers 56 metabolic disorders.

CNV Analysis

A blood sample (5 milliliters) in a tube with EDTA from each patient was analyzed by the aCGH microarrays technology Neuroarray (Genetadi, Bilbao, Spain). This technology is able to detect more than 900 regions associated to mental idiopathic and other neurodevelopment illnesses. To calculate the detection rate of the present study we determined the percentage of genetic defect carriers whilst the diagnostic yield is only referred to the percentage of patients carrying a clinically relevant CNV.

Clinical relevance of detected CNV was assessed by accessing to database through the Ensemble Resources as DECIPHER (Database of Chromosomal Imbalance and Phenotype in Humans), the ISCA database [<http://dbsearch.clinicalgenome.org/search/>], and the OMIM [Mendelian Inheritance in Man].

Results

In the present study, we identified (Table 1) a genetic defect in 97 patients (80% of our sample), where 86 cases (71 %) have a cryptic chromosomal abnormality: 72 CNVs on autosomal chromosomes and 14 on X chromosome, 5 patients (4%) were Fragile X syndromes (FRAXA) and 6 cases (5%) showed a metabolic disturbance. It was not possible to detect a genetic defect in 24 patients (20%) (Figure 1).

N		%	DIAGNOSIS	
86	72	71%	autosomal chrom.	cryptic chromosomal abnormality
	14		X chrom.	
5		4%	fragile X syndromes (FRAXA)	
6		5%	metabolic disturbance	
24		20%	no genetic defect	
121			TOTAL	

Table 1

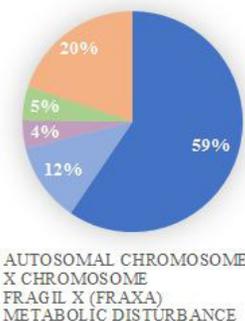


Figure 1: General Results.

The detection rate of our diagnosis protocol was 80% to reach to a diagnostic yield of 71% using the aCGH. There is a predominance of alterations in autosomal chromosomes (83%) with respect to the sex chromosomes (17%). Regarding to sex chromosomes, alterations are more frequent in the X chromosome than in chromosome Y in a relation of 12:2 respectively. By delving into the autosomal causes, we observed that chromosomes with a greater involvement are chromosome 17 (14 cases), chromosomes 2 and 9 (9 cases) and chromosomes 3 and 6 (Figure 2).

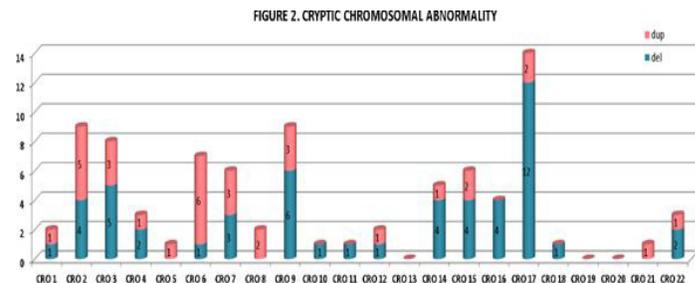


Figure 2: Cryptic Chromosomal Abnormality.

On the other hand, there is a predominance of microdeletions over microduplications (52 vs. 34 respectively) being the 17q microdeletion the most frequent cryptic chromosomal abnormality detected in our sample (10 cases; 13 % of total detected CNVs). There are additional cryptic copy number alterations without clinical

cal relevance (because they were previously reported in a normal population being catalogued as “benign CNVs”) which were identified in 11 cases of our sample (9%). Curiously, there is also a predominance of benign CNVs in the chromosome 17 (8 subjects).

Fragile X Analysis

The numbers of CGG repeats less than 50 were detected in 116 cases. CGG repeats in a full mutation range (more than 200 CGG) were detected in 5 male patients. All these patients showed a clinical phenotype coincident with the Martin-Bell phenotype.

Metabolic Findings

Metabolic defects were detected in 6 of 121 patients (5%):

- Maple Syrup Urine Disease (MSUD)
- Phenyl Keton Uria (PKU)
- Urea Cycle Disorders (UCDs).

Discussion

Chromosomal abnormalities are the most common cause of intellectual disabilities in our patients. Coincident with our results is important to note that other recent scientific studies have detected that 40% of the chromosomal rearrangements apparently balanced using karyotype or FISH, end-up resulting in an unbalanced micro rearrangement after a high-resolution analysis using aCGH microarrays [21,25,26].

Microdeletions were the most frequent type of chromosomal defects detected in our sample suggesting that microdeletions might be more pathogenic than microduplications. This is also consequent with the published results by other authors on this regard [24,27]. In this sense, it is curious to note that the most frequent non-pathogenic cryptic rearrangements we found in our sample are represented by microduplications.

Among the 86 cases with probably pathogenic CNVs, there is a predominance of CNVs which have been previously associated with intellectual disabilities syndromes (MR-associated CNVs) being coincident with some Decipher known syndromes [36]. On the other hand, there are 11 cases with CNVs which are currently of uncertain clinical significance. Due to the characteristics, inherent to our sample (institutionalized adult patients) it was not possible to analyze their inheritance in a family study.

Nevertheless, neither of them has been previously described in normal population nor associated with Decipher known syndromes (<https://decipher.sanger.ac.uk/syndromes#syndromes/overview>). To understand the clinical relevance of a CNV we have to consider many standardized criteria together with the particular clinical evaluation. In addition of being a CNV that have not been observed among healthy individuals, in the first place, we have to consider the CNV size. The larger the CNV the more likely to encompass functional DNA sequences with an impact over the clinical phenotype [37]. In our study, we consider to analyze in depth

as MR-associated CNV those CNVs with more than 100 kb which represent the 88% in our sample.

However, it is important to take into account that the relevance of the gene content might be not only related to the size which mean that quantity of genes is not the unique factor to consider, because quality also matter. There are shorter CNVs containing few genes but having a critical relevance regarding to the analyzed phenotype and in this sense, they might be more relevant than larger CNVs which have several non-relevant DNA sequences. That means that both quantity and quality should always be carefully assessed in order to decide the real significance of each analyzed CNV [38].

Another relevant criterion to consider is the inheritance using family information. In our study, we have 2 families with affected relatives sharing the same CNV which is smaller than 50 kb in both cases. There are 2 affected brothers with the same CNV of 13,3 kb in chromosome 16 (microdeletion at 16q24.1) in one of the families and two affected relatives (brother and sister) in the other family showing an 11, 8 kb CNV at chromosome 14 (microdeletion at 14q22.1) [39,40].

Regarding to the gene content, it is curious to mention that the CNV at chromosome 14 affects the gene PTGER2. This gene codes for the EP2 receptor subtype of prostaglandin E2 (PGE2) functioning as a regulator of several inflammatory and neuroprotective processes. In animal models (knockout mice), it was determined that PTGER2 acts as a regulator of synaptic transmission in the hippocampus [41]. The other CNV at 16q24.1 affects the gene ATP2C2 which is a known candidate gene to have an important neurobiological significance [42].

Our study gives additional support to affirm that chromosomal microarray has to be included as the first clinical diagnostic test for patients with intellectual disabilities. This statement is in concordance with a consensus recently recommended by other researchers worldwide [43,44]. We propose to use a different analytical algorithm for Intellectual Mental Retardation (IMR) where the comparative genomic hybridization microarrays (aCGH) might be used as the first option after clinical valuations.

Conclusion

The present study confirms that genetics is an important factor to take into account in front of severe/profound mental retarded patient with dysmorphic pattern. According with that, CNVs are highly represented in patients with intellectual disabilities and dysmorphic pattern, being the most relevant genetic cause present in our population. The most common CNVs detected in our study related to intellectual disabilities are the microdeletions. The 17q microdeletion syndrome is distinguished as the most prevalent syndrome present in our patients. According to our findings, we consider that it is highly recommended to use the aCGH microarray technology as the first analytical test to carry out as part of the diagnostic protocol of MR patients.

Limitations of present study

Due to the particularities of the subjects included in the present study: institutionalized adult patients with a mean age of 45 years and a mean time of institutionalization of 19.7 years, there was not possible to carry out the corresponding familial testing analysis for more of them.

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