

## Etiology and the Genetic Basis of Intellectual Disability in the Pediatric Population

Višnja Tomac<sup>1,2</sup>, Silvija Pušeljić<sup>1,2</sup>, Ivana Škrlec<sup>3</sup>, Mirna Anđelić<sup>3</sup>, Martina Kos<sup>1</sup>, Jasenka Wagner<sup>3</sup>

<sup>1</sup> Pediatric Clinic, Clinical Hospital Centre Osijek, Osijek, Croatia

<sup>2</sup> Department of Pediatrics, Faculty of Medicine Osijek, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

<sup>3</sup> Department of Medical Biology and Genetics, Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Osijek, Croatia

Corresponding author: Višnja Tomac, MD - [visnja.tomac@yahoo.com](mailto:visnja.tomac@yahoo.com)

### Abstract

Intellectual disability/mental retardation (ID/MR) is defined as incomplete mental and cognitive development present before the age of 18. There are number of pre-natal and post-natal risk factors that can cause ID/MR but 25 %-50 % of all have genetic causes. In the general population, the prevalence of ID/MR is about 2 %-3 %. Use of standard cytogenetic methods analysis of chromosomes (GTG banding) and FISH (Fluorescent in Situ Hybridization) reveals only a small number of causes, but when using new molecular genetics techniques (like chromosomal microarray and next generation sequencing), the rate of causes of ID/MR is increased and new candidate genes for ID/MR have been discovered. Establishing a diagnosis of ID/MR is important for the patient and it provides genetic counseling for parents.

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### Definition and prevalence of ID/MR

Intellectual disability/ mental retardation (ID/MR) is defined as a disability characterized by significant limitations in intellectual functioning and in adaptive behavior; condition covers everyday social and practical skills and begins before the age of 18. Intellectual functioning, also called intelligence, refers to

general mental capacity, such as learning, reasoning, problem solving and so on. Adaptive behavior is the collection of conceptual (language and literacy), social (interpersonal skills, social responsibility) and practical skills (activities of daily living and personal care, occupational skills, healthcare) that are learned and performed by people in their everyday living (1).

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**Table 1.** Environmental and genetic causes of intellectual disability

Prenatal factors	Perinatal factors	Postnatal factors
<i>Genetic:</i> chromosomal abnormalities cryptic chromosome abnormalities deletions/duplications contiguous gene syndromes monogenic diseases	prematurity low birth weight asphyxia	sepsis/meningitis, encephalitis (HSV 1/2) various multifactorial causes (poverty and cultural factors)
<i>Environmental:</i> infections (toxoplasmosis, syphilis, rubella, cytomegalovirus and HIV infections) mother disease (diabetes) teratogenic factors (drugs and radiation)		
<i>Metabolic:</i> neonatal hypothyroidism		

The intelligence quotient test (IQ test) is a major tool in measuring intellectual functioning, which is the mental capacity for learning, reasoning, problem solving and so on. A test score below or around 70 or as high as 75 indicates a limitation in intellectual functioning. IQ testing became the way to define groups and classify people within them (1). According to IQ testing, ID/MR is categorized as: mild (IQ 50 - 55 to 70), moderate (IQ 35 - 49 to 50 - 55), severe retardation (IQ 25 - 20 to 35-40), or profound retardation (IQ below 20).

The prevalence of ID/MR varies considerably due to the different criteria and methods used in the diagnosis. This problem is present in 2 % to 3 % of the children's population, especially because 5 % to 10 % of children have motor impairment, isolated speech and language delay, severe primary sensorial deficits and pervasive disabilities. ID/MR is more frequent in countries of lower socioeconomic status due to increased incidence of anoxia, birth trauma and newborn brain infections (2). The prevalence of mental retardation in developed countries is thought to be 2% to 3%. The prevalence of mild ID/MR more often depends on external environmental factors (level of maternal education, access to education, opportunity and access to healthcare), while the prevalence of severe ID/MR is relatively stable (3).

Diagnosis is highly dependent on a comprehensive personal and family medical

history, a complete physical examination and a careful developmental assessment of the child. When diagnosing ID/MR, it is very important to know how it is defined and classified.

### Etiology and epidemiology of ID/MR

The etiology of ID/MR has heterogeneous environmental and genetic causes, summarized in Table 1 (4, 5). Prenatal factors are environmental (mother infection in pregnancy such as rubella infections, syphilis, toxoplasmosis, cytomegalovirus and HIV infections), teratogenic (the use of drugs such as thalidomide, phenytoin and warfarin sodium in early pregnancy, radiation), chromosomal abnormalities (e.g. trisomy 21), cryptic chromosomal abnormalities (deletions or duplications) and genetic mutations. Perinatal factors are prematurity and asphyxia, while postnatal factors are sepsis, meningitis, encephalitis (commonly caused by HSV 1/2) and various multifactorial causes (poverty and cultural factors).

Genetic factors are thought to cause ID/MR in about 25% to 50% of cases (6). Specifically, genetic factors are estimated to be the cause of moderate and severe ID/MR (IQ<50) in 0.3 % to 0.5 % of cases, of mild ID/MR (IQ ranging from 50 to 70) in 1 % to 3 % of cases and of severe ID/MR in 25 % to 50 % of cases (7).

Based on the symptoms' presentation, ID/MR is divided into two groups: syndromic and non-syndromic ID/MR. In non-syndromic ID/MR, the only pathological manifestation is cognitive deficit, and there are no changes in phenotype and no associated anomalies of organ systems. It can be inherited in three ways: autosomal recessive, autosomal dominant or X-linked mode. Syndromic ID/MR is related to phenotypic dysmorphism (craniofacial, skeletal), growth changes, neuromuscular changes and metabolic diseases (8).

#### *Chromosomal abnormalities*

Aberrations in the autosomal chromosome number in live-born babies are restricted to aneuploidies. These abnormalities represent about 10 % of the ID/MR that can be detected with conventional cytogenetic methods (9). The majority of cases involve trisomy 21 with a prevalence of 1 to 700, which is clinically expressed as Down syndrome (10). Other rare chromosomopathies include trisomy 13 (Patau syndrome) with a prevalence between 1 in 5,000 and 1 in 29,000 live births (11), trisomy 18 (Edwards syndrome) with a prevalence of 1 to 3600 and 1 to 8500 (12), and they are usually lethal in the first week of life. Monosomy of any autosomal chromosome is lethal in the earliest stage of embryonic life. There are autosomal structural abnormalities such as Wolf-Hirschhorn syndrome (microdeletion 4p) with a prevalence of 1 to 50,000 (13), Cri du Chat syndrome (microdeletion 5p) with a prevalence of 1 to 50 000 (14) and sex chromosomal aneuploidies such as Klinefelter syndrome (47,XXY) with a prevalence of 1 in 500 to 1,000 newborn males (15).

#### *Contiguous gene syndromes*

Contiguous gene syndromes are disorders caused by chromosomal abnormalities, such as deletions and duplications, which result in an alteration of normal gene dosage. For most autosomal loci, deletion causes a reduction of gene dosage to structural and functional monosomy. Haploinsufficiency for specific genes in the critical interval is implicated for

del(7)(q11.23q11.23) in Williams syndrome, for del(8)(q24.1q24.1) in Langer-Giedion syndrome, del(17)(p13.3) in Miller-Dieker syndrome, and for del(22)(q11.2q11.2) in DiGeorge syndrome and velocardiofacial syndrome (16).

#### *Genomic imprinting*

Genomic imprinting is a situation in which there is gene expression from only one of the two alleles inherited from each parent, and it is based on epigenetic modifications of specific allele, such as histone acetylation/methylation and DNA methylation (17).

The deletion of a chromosome segment containing the active allele of an imprinted gene results in structural monosomy but functional nullisomy (e.g., paternal del(15)(q11.2q13) in Prader-Willi syndrome and maternal del(15)(q11.2q13) in Angelman syndrome). Uniparental disomy for the homologue containing the inactive allele results in structural disomy but functional nullisomy (e.g., maternal disomy 15 in Prader-Willi syndrome and paternal disomy 15 in Angelman syndrome).

#### *Idiopathic ID/MR*

Current research has been directed to clarify the genetic base of what was accepted as "idiopathic ID/MR". The most prevalent structural variations in the human genome are copy number variations (CNVs), which appear predominantly in the subtelomeric regions. Genomic variations are a frequent cause of miscarriage, congenital anomalies (CA) and intellectual disability (ID) (18).

Pathogenic CNVs have been detected in 10 % to 15 % of patients with idiopathic ID/MR, especially with use of microarray technology. Most of CNVs are de novo mutations, but there are also rare inherited mutations with unknown significance (19).

Over the last few years, cryptic chromosomal anomalies, particularly subtelomeric and interstitial rearrangements (including microdeletions as well as balanced translocations and other chromosomal

aberrations) less than 3–5 Mb, have emerged as a significant cause of "idiopathic ID/MR" (20-22).

About half of all segmental aneusomies are found on subtelomeric and terminal regions of chromosomes that are gene-rich, and they are responsible for 5% to 7% of all cases of ID/MR (23, 24).

### *Monogenic causes*

X-linked mental retardation (XLMR) is a common cause of monogenic intellectual disability, because most of genes causing ID/MR are found on the X chromosome. X-linked forms of mental retardation are estimated to cause 10-20% of all inherited cases of ID/MR. There is a higher prevalence of ID/MR among males relative to females (1.8 in 1000 males; carrier females 2.4 in 1000). However, female carriers may manifest mild symptoms, due to a skewed X-inactivation (25).

Based on symptoms' presentation, XLMR can be divided into three groups: 1) syndromes - characterized by multiple congenital anomalies (phenotypic dysmorphism, organ anomalies); 2) neuromuscular disorders - epilepsy, dystonia, spasticity, muscle weakness and so on without malformations and 3) nonspecific conditions (MRX) - isolated ID/MR is the only clinical manifestation. There are 215 XLMR conditions divided according to their clinical presentation: 149 with specific clinical findings, including 98 syndromes and 51 neuromuscular conditions, and 66 nonspecific forms (26).

Fragile X syndrome (FRAXA, OMIM 309550) is the most common form of syndromic XLMR (20 % of all XLMR cases), with a prevalence of approximately 1:5000 males, and causes intellectual disability in about 1 in 8000 females (27). Affected individuals have a folate-sensitive fragile site in the region Xq27.3, associated with an expansion of a trinucleotide repeat (CGG) in the 5'-noncoding region of a gene that encodes an RNA binding protein termed FMR1. Individuals with fragile X syndrome have a loss-of-function variant of FMR1 caused by an increased number of CGG trinucleotide repeats

(typically >200) accompanied by aberrant CpG methylation of FMR1 (28).

Another common gene is MECP2 (methyl CpG binding protein 2 (OMIM 300005) on chromosome Xq28, which causes Rett syndrome, affecting approximately 1 in every 10,000–15,000 females worldwide (29). But it is also identified in the clinical spectrum seen in males with severe neonatal-onset encephalopathy or with X-linked intellectual disability associated with psychosis, pyramidal signs, parkinsonian features and macro-orchidism (PPM-X syndrome; OMIM 300055) (30).

## **Evaluation and Testing**

The clinical geneticist has an important role in the evaluation of patients with intellectual disability and in making decisions about further genetic testing. Evaluation includes physical examination and the collection of family history information. The physical exam should focus on dysmorphological and neurological evaluation, congenital malformations, somatometric measurements and behavioral evaluations. In all patients with neurological symptoms, such as epilepsy and macro/microcephaly, neuroimaging studies - MRI (magnetic resonance imaging) should be performed for evaluation of brain malformations. If there are signs of metabolic disease, metabolic tests should be done (organic acid in urine, amino acids in serum, lactate, pyruvate) (31).

When investigating a patient with ID/MR, with or without dysmorphic features, the initial analysis several years ago usually began with cytogenetic testing (GTG-banding).

GTG banding (G-banding with Trypsin/Giemsa) is used for the detection of aneuploidy (abnormal number of chromosomes) and the identification of structural aberrations: deletions and translocations in chromosomal rearrangements only larger than 5–10 Mb. The overall yield of routine cytogenetic testing is 3.7 % (32).

Fluorescent in situ hybridization (FISH), using location specific probes, detects

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submicroscopic alterations less than 5 Mb that cannot be observed using standard cytogenetic tests (GTG-banding). Today this method is used when a specific syndrome is suspected with high frequency in the general population (e.g., DiGeorge/velocardiofacial syndrome, Williams Beuren syndrome). The yield of FISH screening on patients with moderate to severe ID/MR is 6.8 % (33).

Candidates for subtelomere screening are patients with ID/MR and two or more dysmorphic features (mostly facial), congenital organ abnormalities, skeletal abnormalities, positive family history and pre/postnatal poor growth/overgrowth (34).

Several assays are currently available to detect subtelomeric rearrangements, but subtelomeric FISH and subtelomeric MLPA have been the most frequently used. MLPA results needed to be confirmed using other more accurate techniques such as FISH or aCGH (35).

With the introduction of comparative genomic hybridization on microarrays it is possible to screen the entire genome for evaluation of deletions and duplications of specific DNA sequences. Comparative genomic hybridization on microarrays (Array Comparative Genomic Hybridization - aCGH) and the technical basis of the method was first published in 1997 (36). Detection of subtle submicroscopic changes in a number of copies of DNA less than 1Mb is possible using different platforms. With the application of aCGH in patients with ID/MR it is possible to determine etiology in 20% of patients with normal karyotype and subtelomere screening with MLPA (36).

Copy number variations (CNVs) are the most prevalent structural variations in the human genome, which appear largely in the subtelomeric regions and can be detected by aCGH. ID/MR is associated with variable sizes of CNVs (18).

A disadvantage of the aCGH is that the identification of de novo CNVs of uncertain significance and unreported CNVs can be challenging to interpret. CNVs should be listed as benign or pathogenic, or reported as variants

of unknown clinical significance (37). Pathogenic variants are detected in 15 % to 20 % of ID/MR patients (37, 38). Not all CNVs are fully penetrated or cause a spectrum of phenotypes, including intellectual disability, autism, schizophrenia, and dimorphisms. Such CNVs can pose challenges to genetic counseling. More variants of uncertain significance are found with higher density arrays (38). Sometimes, variants of unknown significance can be resolved by trio testing (mother, father and proband). Interpretation of those variants is very comprehensive and challenging, and demands bioinformatics and clinical knowledge.

Next-generation sequencing (NGS) is DNA sequencing technology that sequences all genes in one genetic test. Exome sequencing analyzes all exons of protein coding genes in the genome known as a cause of the diseases (clinical exome sequencing) or all of the genes in the genome (whole genome sequencing). NGS in a clinical setting opens up possibilities for discovering the genetic contribution for a large percentage of ID/MR individuals at the first onset of symptoms and the possible opening up of pathways for therapeutic interventions (37). NGS is progressively being set up in clinical laboratories for the diagnosis of ID/MR because of a higher diagnostic yield and devaluation in costs. Many studies revealed the usefulness of using an integrative approach to examine genotype-phenotype variability (37, 39, 40).

Whole exome sequencing (WES) is an impressive tool for identifying clinically undefined forms of ID/MR, especially when aCGH identified a de novo CNV of uncertain significance (37).

The vast majority of benign variants are single base pair substitutions. With better coverage depth, WES is adequate for the detection for close to all (99.7 %) pathogenic variants (41). CNV analysis is still an active area of research in NGS variant analysis and has long been important in ID research. CGH microarrays can only detect unbalanced structural variants, while apparently balanced chromosomal rearrangements occur in 1.54 % of live births and contribute to 6 % of abnormal phenotypes including ID (42). Whole

**Table 2.** Advantages and disadvantages of genetic methods for diagnosing intellectual disability

Method	Advantages	Disadvantages
GTG (G banding with Trypsin and Giemsa)	Whole genome analysis Detection of unbalanced and apparently balanced chromosomal rearrangements	Time consuming Small resolution (5 to 10 Mb)
FISH (fluorescent in situ hybridization)	Detection of unbalanced and apparently balanced chromosomal rearrangements and mosaicism Detection of small deletions and duplications	Time consuming Small resolution (depend on the size of FISH probe, 30 to 100 kb)
MLPA (multiplex ligation probe amplification)	High-throughput Simultaneously analyses of several samples Multiplex technique (study of several regions of the human genome in a single reaction) Low cost	Not whole genome analysis Sensitive to PCR inhibitors
aCGH (microarray comparative genomic hybridization)	Whole genome analysis High resolution (up to 40 kb)	Impossibility of detection of apparently balanced chromosomal rearrangements and mosaicism CNVs of unknown significance in clinic
NGS (next generation sequencing)	Whole genome analysis High resolution (covering all coding variation) Single strand sequencing	CNVs of unknown significance in clinic Expensive

genome sequencing (WGS) has the potential to uncover all forms of genetic variation in one test, and offers a higher diagnostic yield (43). In the study of Harripaul et al. of patients with severe ID, a diagnostic yield of 42 % was observed (42) which is a significant improvement over the diagnostic yield obtained by microarray, gene panels or WES (44). A summary of genetic methods used in diagnosing ID/MR is presented in Table 2.

## Discussion

Defining the cause of intellectual disability/mental retardation (ID/MR) presents a diagnostic challenge. Mental retardation is present in about 1 % to 3 % of individuals in the general population, but there are many cases that cannot be explained despite novel technology and clinical investigations (24). Genetic factors are involved in many of the idiopathic cases of ID/MR. This conclusion is

based on the fact that these patients often show signs such as dysmorphic features, growth retardation and malformations, or have a family history of mental retardation (6,7).

The genetic heterogeneity of intellectual disability requires genome wide approaches, including the detection of chromosomal aberrations by chromosomal microarrays and whole exome sequencing adequate for discovering single gene mutations (45).

For individuals with idiopathic ID/MR, autism spectrum disorders, or multiple congenital anomalies, chromosomal microarray analysis (CMA) is recommended as the first-line diagnostic test since it offers a much higher diagnostic yield (15 % to 20 %) compared with G-banded karyotype analysis (3 %) (38,42).

Despite those modern technologies, the genetic etiology of 80 % to 85 % of patients still remains unknown. NGS-based testing (targeted

multigene panels, whole exome sequencing or whole genome sequencing) for these cases, has a great potential to obtain diagnosis (44).

The vast majority of individuals with ID/MR currently receive no molecular diagnosis, which is a shortcoming that significantly impacts health and life span. There is also a strongly negative correlation of survival with the severity of ID (46). It is important to emphasize that knowing which genes carry mutations that cause ID/MR can have huge benefits for diagnosis in clinics, and can lead to better understanding of each patient's health issues, more appropriate care and treatment, improved overall health and life span, and appropriate counseling and planning for families.

### Abbreviations

ID/MR - intellectual disability/mental retardation; GTG- G-banding with Trypsin/Giemsa; FISH - Fluorescent in situ hybridization; MLPA-Multiplex Ligation dependant Probe Amplification; aCGH - Array Comparative Genomic Hybridization; NGS - Next generation sequencing; CNVs - copy number variations; XLMR - X linked mental retardation; XLID - X linked intellectual disability; IQ- intelligence quotient test; FRAXA-fragile X syndrome; MRI - Magnetic resonance imaging; CA - congenital anomalies.

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### Transparency declaration

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