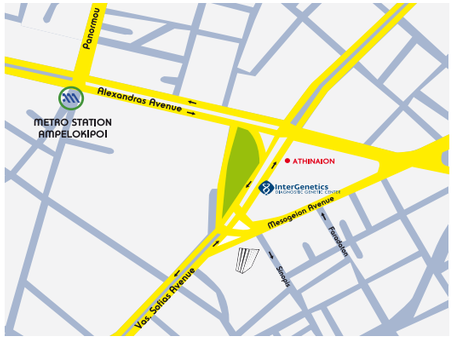

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Molecular genetic testing for X-linked mental retardation – testing of 14 genes

Apart from the Fragile X syndrome, which is the most common, more than 14 genes on the X chromosome are known to be involved in X-linked mental retardation (affecting males).

The MLPA technique is applied, which allows to detect deletions or duplications of exons of the genes: MECP2, ARX, ARHGEF6, TM4SF2, RPS6KA3, IL1RAPL1, OPHN1, GDI1, PQBP1, DCX, PAK3, AGTR2, ARHGEF6, SLC6A8, located in various positions on the X chromosome and which are detected in ~5% of patients with X-linked mental retardation.

Genomic analysis – whole exome sequencing in mental retardation

Sequence analysis of all known human genes (whole exome sequencing - WES), through new generation sequencing technologies (next generation sequencing - NGS) is the new genetic super- weapon, as it is theoretically in a position to uncover the molecular basis for any genetic disorder in an affected individual, which has not been diagnosed by other available conventional genetic testing options. This test is completely different from other types of currently known genetic testing, in terms of the number of genes that are analyzed simultaneously.

Now, this new powerful technology allows high-throughput analysis of all ~22,000 human genes (whole exome sequencing) or, alternatively, the simultaneous analysis of hundreds of genes (gene panels) associated with different categories of diseases with a genetic etiology, such as mental retardation, neuro-developmental disorders, undiagnosed syndromes and neurogenetic disorders, autism, etc., which may be due to abnormalities in many different genes.

The test is based on the method of next generation sequencing (NGS) and utilizes a special Genome Analyzer instrument together with complex and highly specialized software tools. The test is generally completed within 4-5 months.

The test is highly sensitive and complex. Therefore, the analysis and the clinical evaluation of the results should be performed by a highly skilled group of clinical and molecular geneticists, ensuring the maximum diagnostic validity for the patient and the family.

Why is genetic testing for mental retardation useful

Unraveling the genetic cause associated with mental retardation is particularly valuable (if not necessary) for the further management of the case by the referring physician. Without this knowledge it is difficult to perform genetic counseling regarding disease progression in the patient, for providing advice regarding reproductive options for the couple but also for assessing the overall impact of the disease in the family.

It is essential, therefore, that genetic testing is recommended and to be in-depth and comprehensive, covering as much as possible all possible alternatives, but always after proper evaluation of the clinical data by a specialist physician and a clinical geneticist.

Genetic counseling

Proper clinical genetic assessment of each case and genetic counseling, both before and following the test, is essential in order to determine the appropriate strategy for laboratory testing and to interpret correctly the concepts of pathological and normal.

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GENETIC TESTING FOR MENTAL RETARDATION

...it is indeed true...

- ✓ *that 1 in 50 people experience a degree of mental retardation*
- ✓ *that the cause is frequently unknown and possibly inherited*
- ✓ *that today we have the means of aiding in the diagnosis...*

Mental retardation in humans is divided into mild, moderate and severe and occurs with a frequency of approximately 2% in the general population, with a usually undiagnosed etiology.

Many forms are inherited within the family, while there is also idiopathic sporadic mental retardation without previous family history, which is the most common type.

Generally, recognizing the cause of mental retardation at the genetic level is difficult, since many different syndromes and hereditary diseases include mental retardation as part of the symptoms.

Modern genetic diagnostics include a series of tests that may unravel the genetic causes of mental retardation in an affected person.
These tests are:

Peripheral blood karyotype

Today, the test is applied mainly, if not exclusively, for the diagnosis of chromosomal syndromes with recognizable clinical phenotype that includes mental retardation. It is possible that the karyotype of an individual reveals a numerical chromosomal abnormality, e.g. trisomy 21 – Down syndrome, an aneuploidy involving the sex chromosomes X or Y in all or a percentage of cells (mosaicism).

It is also possible that an extensive structural imbalance may be detected, involving a deletion or duplication, derived from a translocation or inversion of chromosomal material, possibly of parental origin.

Chromosome analysis is performed utilizing high resolution banding techniques (e.g. RTBG, 550-850 bands) from at least 20 metaphases. In cases of suspected mosaicism, at least 100 metaphases are analyzed.

Molecular testing for microdeletion/ microduplication syndromes

Morphological analysis of the chromosomes cannot reveal submicroscopic imbalances, mainly deletions or duplications, which are particularly prevalent in areas near the ends of chromosomes (telomeres) and has been found to account for 8-10% of the cases involving moderate/severe mental retardation.

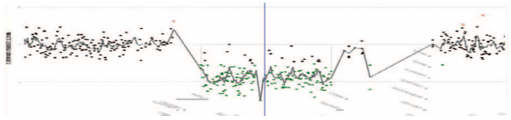
Clinical symptoms that usually accompany these cases include hearing loss, psychomotor problems, growth retardation, dysmorphic features, congenital malformations, feeding difficulties, etc.

If such a syndrome is suspected, diagnosis is aided through the application of the MLPA technique for the analysis of specific microdeletions/ microduplications associated with the syndrome.

Molecular karyotype, high resolution (microarrayCGH)

If the clinical data suggests the need for a massive and detailed testing for genomic imbalances, which might be the cause of mental retardation in the patient, then we apply this relatively new test, which is now internationally recommended as a first-tier genetic test for mental retardation and developmental disorders.

It is applied mainly in patients with a pathological phenotype, which may include mental retardation, with or without constitutive abnormalities, developmental delay, autism, learning problems, etc.



It is an extremely powerful tool for the diagnosis of the cause of many pathological phenotypes, not revealed through the morphological analysis of chromosomes (conventional karyotype). It is also invaluable for the clinical geneticist, so that he may provide detailed genetic counseling regarding both the severity of the disease and the likelihood of recurrence of the problem in the family. Pre and post-testing genetic counseling is highly recommended.

The test is performed utilizing the Agilent 105K microarray CGH platform, validated for clinical diagnostic use, covering the entire human genome at an analytical level of ~100 Kb. The test requires DNA from a peripheral blood sample.

Fragile X syndrome (FRAX)

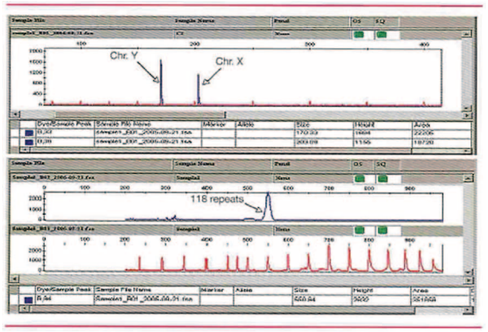
Fragile X syndrome (FRAXA) is the most common genetically-inherited form of mental retardation, occurring mostly in males. As the name suggests, it is related to an increased fragility at the Xq27.3 region of the X chromosome.

Since the region responsible for the syndrome is located on chromosome X, the disease is X-linked (affecting males who have only one X chromosome), while women who have two X chromosomes are carriers and do not usually express the disease. Less than 1/3 of female carriers' exhibit mild to moderate mental retardation, which can often go unnoticed.

The incidence of the syndrome is about 1/5,000 male births, while the frequency of female carriers is about 1/250 - 1/500 women.

The genetic cause of disease is due to an abnormal expansion of the number of (CGG) triplet repeats in the FMR-1 gene, located in the FRAXA region of the X chromosome (Xq27.3).

There are also a small number of patients with moderate mental retardation, caused by an expansion of (CGG) triplet repeats in the FMR-2 gene, located in the nearby FRAXE region.



Generally, a number of CGG triplet repeats greater than 200 is considered pathological.

Genetic testing is applied through the detection of the exact number of CGG triplet repeats in the FMR1 gene and the FMR2 gene (FRAXA and FRAXE) and by methylation analysis of the FMR-1 gene promoter, through a specially designed fluorescent PCR reaction, while Southern blotting is applied whenever it is deemed necessary.

Our laboratory participates successfully in the external quality control scheme organized by the European Molecular Genetics Quality Network (EMQN), which is periodically applied for genetic testing of Fragile X syndrome-FRAXA.