InterGenetics
DIAGNOSTIC GENETIC CENTER

GENETIC TESTING FOR NEUROGENETIC DISORDERS

- ✓ *wide spectrum of genetic tests, for many common and rare neurogenetic disorders*
- ✓ *comprehensive testing utilizing a combination of techniques, leading to full detection of pathological mutations*

Spinal muscular atrophy (SMA) is a relatively common neuromuscular disease. It is inherited as an autosomal recessive genetic disease and is caused by mutations in the SMN1 (survival motor neuron 1) gene on chromosome 5q13.

It affects primarily muscles of the extremities (feet, hands) and muscles of the trunk (lungs, swallowing reflexes, etc.). It is one of the most common genetic causes of infant mortality, with an incidence of 1/10,000 births and the carrier frequency in the general population is ~1/50.

In humans, there are two genes called survival motor neuron 1 and 2. The homozygous (both chromosomes) absence of functional copies of the SMN1 gene (which is the main functional gene) leads to the onset of disease in >95% of patients, while the remaining cases are due to point mutations of the gene on the another chromosome.

However there is another form of the gene called SMN2, which however produce much lower amount of functional protein than the SMN1 gene. A key way to determine the clinical diagnosis of the severity of the disease is the simultaneous and accurate determination of the number of copies of the SMN2 gene.

An increase in the number of copies of this gene (>3-4) leads to a larger amount of SMN2 protein produced and thus offsets somewhat the loss of functional SMN1. Patients (with no functional SMN1) having one or two copies of SMN2 have a more severe phenotype, while those with three or more copies of SMN2 have milder disease symptoms.

Testing includes full deletion/duplication analysis of SMN1 and SMN2 exons and of the wider chromosomal region 5q13 using the MLPA technique, which allows us to safely diagnose carriers and affected individuals. Moreover, we apply mutation analysis of all exons of the SMN1 gene and intron-exon junctions, by automated bi-directional fluorescent DNA sequencing, revealing mutations in the remaining 4-5% of patients harboring a heterozygous deletion on other allele.

Huntington's disease is an incurable inherited neuro-degenerative disease, which is expressed gradually and is characterized by both emotional and psychiatric abnormalities and also from behavioral abnormalities, loss of mental and brain function and motor dysfunction. It is inherited as an autosomal dominant disease, meaning that all persons who inherit a mutated copy of the gene will develop the disease at some point in life. The classic symptoms of the disease, which occurs with a frequency of 1/15,000 - 1/30,000 individuals, include chorea, or otherwise involuntary repetitive irregular movements that occur on the face, arms, and the gradual loss of thinking and acquired mental faculties (dementia).

The disease is the result of an expansion of the triplet of bases (CAG) in the IT15 gene (on chromosome 4p16.3). In healthy individuals the number of triplet repeats is up to 36, while in those suffering from the disease this triplet sequence is present in more than 36 consecutive repetitions. Number of (CAG) repeats between 36-41 usually result in reduced penetrance of the disease, which means that the person may or may not develop the disease.

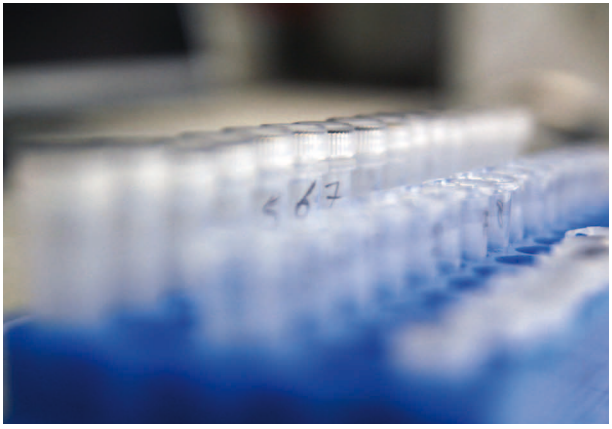
A special fluorescent multiplex polymerase chain reaction (PCR) is applied, which detects the exact number of (CAG) repeats (±1) in the gene IT15.

Muscular dystrophy Duchenne/Becker is a genetic disease of the neuromuscular system, typically manifested by progressive weakness in muscles and usually results in complete disability and death. It is caused by mutations in the dystrophin (DMD) gene, one of the largest human genes, and is inherited X-linked recessive manner affecting only boys. The incidence of the disease is about 1/3,500 male births. Becker muscular dystrophy is also due to genetic lesions in the same gene, but is a clinically milder form to Duchenne and manifests later in life. The most frequent mutations are deletions or duplications of one or several exons the DMD gene, associated with ~75-80% of Duchenne and ~85% of Becker muscular dystrophy. The remaining percentage (15-25%) are point mutations, which are distributed randomly and is extremely difficult if not impossible to detect.

Note that approximately 2/3 of affected boys have inherited the mutation from their mother (who is a carrier) while in 1/3 of the cases the mutation is de novo, appearing for the first time in the patient (without the mother being a carrier). It is therefore important to perform molecular genetic testing in patients, not only to confirm the diagnosis, but mainly for the ability to test other family members and identify female carriers of the disease.

Sometimes, it is possible to perform indirect molecular genetic testing through genetic linkage studies, without knowing the exact mutation in the DMD gene, but this requires that there are 'informative' family members available for testing.

Deletions or duplications of DMD gene exons are detected by applying the MLPA technique, which allows us to analyze all 80 exons (79 exons + alternative exon DP427C) of the gene (Xp11.2). Furthermore, we apply full mutation testing of all exons of the DMD gene by automated bi-directional fluorescent DNA sequencing, thus uncovering mutations in patients or female carriers who are negative for deletions/ duplications.



Friedreich's ataxia is the most common form of hereditary ataxia with an incidence of about 1 in 50,000 people. it is inherited as an autosomal recessive neurodegenerative genetic disease characterized primarily by progressive limb ataxia, gait ataxia, loss of deep tendon reflex, loss of sense of position and is often accompanied by hypertrophic cardiomyopathy and diabetes. The onset of symptoms usually begins in adolescence and the development of the disease is complete by 25 years of age, and clinical diagnosis follows the criteria of Harding.

In >98% of cases the disease is caused by a pathological expansion of a (GAA) triplet in both copies of the frataxin gene on chromosome 9q13. The carrier frequency of an abnormal (GAA) expansion in the general population is about 1/90, but in different populations the frequency may differ. In normal subjects the (GAA) triplet is repeated from 8 to 33 times, while expansion of the triplet >90-1,300 times is associated with pathological expression of the gene (and is considered a mutation). Expansions in the range of 35-100 times are considered to be a pre-mutation, which may be further amplified to a full mutation in subsequent generations.

Genetic testing is performed through a special fluorescent multiplex PCR reaction and a specific triplet-repeat PCR, detecting the exact number of repeats as well as the existence of an abnormal expansion of the (GAA) triplet.

Kennedy disease (SBMA) is due to mutations in the gene coding for the androgen receptor (AR), located at chromosome Xq11-q12. This disease is also a relatively rare (1/50,000) neuromuscular genetic disorder, which occurs gradually and affects only men, causing the loss of motor neurons of the extremities and of the head and neck. The disease leads to muscle weakness and paralysis, and affected individuals often exhibit gynecomastia, testicular atrophy and infertility. All patients have an abnormal increase in the number of (CAG) repeats in exon 1 of the AR gene. More than 38 repeats are considered pathological, while the normal number is up to 34 repetitions. Until now, all mothers with affected male children have been found to be carriers of an abnormal elongation of the (CAG) triplet.

A specially designed fluorescent multiplex PCR is applied, which detects the exact number of (CAG) repeats (±1) of the AR gene.

The spino-cerebellar ataxias (SCA) comprise a heterogeneous group of neurodegenerative genetic diseases with clinical symptoms that often are quite similar. They are all inherited as an autosomal dominant genetic disorder and patients present with a phenotype affecting the coordination of body movements, gait ataxia, and other similar symptoms. About 15 different types of spino-cerebellar ataxias have been described, based on the corresponding genes and chromosomal regions involved. Due to the often observed similarity of symptoms, clinical diagnosis of the precise type of SCA is difficult and is based on the results of molecular genetic testing. The majority of mutations usually involve an abnormal expansion of a (CAG) triplet located in the corresponding gene, and this leads to the expression of SCA1, SCA2, SCA3, SCA6, SCA7 or SCA 12. SCA 10 is caused by abnormal expansion of the (ATTCT) sequence of bases.

A reliable final diagnosis is based on the accurate detection of the number of repeats in the corresponding gene, which allows us to

determine whether someone is affected by this type of ataxia disorder.

Molecular genetic testing is performed by applying a special fluorescent multiplex PCR and triplet-repeat PCR reaction, which detects the exact number of (CAG) repeats (±1) and of the ATTCT repeat as well as the expansions of these two sequences in the corresponding gene.

Myotonic dystrophy type 1 and 2, (DM1 and DM2) is the most common form of muscular dystrophy which occurs in adults, with an incidence of about 1/8,000. It is characterized by muscle weakness and especially of myotonia, which causes chronic muscle spasm. Myotonic dystrophy is also characterized by cataracts, premature hair loss in the area of the forehead, heart disorders, etc. There are two main types of myotonic dystrophy: type 1 and type 2 (DM1 and DM2), and their symptoms overlap, although DM2 is milder than type of DM1.

The muscle weakness associated with DM1 particularly affects the legs, hands, neck and face. Muscle weakness in DM2 includes the muscles of the neck, shoulders, elbows and hips. The two types are due to mutations in different genes and are both inherited in the autosomal dominant manner.

DM1 is due to a mutation in the myotonic kinase (DMPK) gene, located on chromosome 19q13.3.

The pathological mutation is an increase in the number of (CTG) triplet repeats in the DMPK gene.

The normal number of (CTG) repeats is 5-37 in both copies of the gene, while in patients this number increases significantly, often to >1,000, thus affecting the normal function of the protein produced by the gene.

If a person has 38-49 repetitions he will not develop the disease, but there may be an increased risk for his children to inherit the disease.

DM2 is rarer and is caused by a similar increase in the number of (CTG) triplets in the ZNF9 (CNBP) gene, located on chromosome 3q21.3.

A special fluorescent multiplex PCR and triplet-repeat PCR reaction is performed, which detects the precise number of (CTG) repeats (±1) as well as pathological expansions of the triplet in the DMPK and ZNF9 genes.

Charcot-Marie-Tooth (CMT) is one of the most common hereditary neurological diseases and is divided into different subtypes (CMT1, CMT2, CMT4 and CMTX). It constitutes a broad group of diseases caused by mutations in several genes that regulate normal functioning of peripheral nerves.

A typical feature of the disease is the loss of muscle tissue and touch sensation, mainly in the legs but at an advanced stage is also manifested in the upper extremities. The disease is divided into different types, each with different modes of inheritance, age of onset of the symptoms and severity as well as variable disease progression.

The most common and 'classical' form of CMT is the CMT1A type (incidence 1-2/10,000), which is inherited as dominant disorder and is caused by mutations in the PMP22 gene at 17p11.2. CMT1A is due to a duplication of the PMP22 gene in >99% of cases and this duplication is absolutely diagnostic for the disease.

There is also the CMTX1 type, which is an X-linked form of the disease and the second in frequency, covering a proportion ~20% of all cases of Charcot Marie Tooth and >90% of the X-linked form CMTX. It is caused by mutations in the GJB1 gene (Cx32) located on chromosome X and male patients express mainly moderate to severe motor and sensory neuropathy, which may also be accompanied by hearing loss, while females are asymptomatic carriers. Pathological mutations in the GJB1 gene are detected in >90% of patients.

Mutation detection is performed by automated bi-directional fluorescent DNA sequencing of the entire GJB1 gene, coupled to analysis for the presence of deletions/duplications, thus covering >99% of mutations of the disease.

Rett syndrome is caused by mutations of the MECP2 gene on the X chromosome, leading to the manifestation of serious neurological and developmental abnormalities in both males and females. The disease is inherited in an X-linked dominant manner and in females it may manifest as classic Rett syndrome, non-classic Rett syndrome or moderate developmental and mental retardation. It occurs more rarely in males, with particularly severe neonatal encephalopathy and microcephaly, often leading to premature death or mental retardation and psychotic disorders (PPM-X syndrome). The incidence of Rett syndrome in females is ~1/8,500.

Molecular genetic testing is performed through automated bi-directional fluorescent DNA sequencing of all exons of the MECP2 gene coupled to analysis for the presence of deletions/duplications, thus covering >99% of mutations of the disease.

When do we apply molecular genetic testing for neurogenetic disorders

Molecular genetic testing for neuromuscular disorders is applied in a slightly different manner for each disease, depending on the mode of inheritance and the clinical features of the disease.

Especially for the dominant neuromuscular diseases (e.g. Huntington, Charcot-Marie-Tooth CMT1A), molecular genetic testing is mainly applied:

- as a diagnostic test in patients manifesting clinical symptoms, in order to confirm the exact type of disorder and reveal the precise genetic lesion

- as a predictive test, in members of a family with affected relatives, who wish to know if they are at risk of being affected. In this case genetic testing must be preceded by genetic counseling
- in prenatal diagnosis, in cases where a parent is affected and the genetic lesion is already known (a necessary prerequisite), in order to determine whether the fetus is at risk of being affected later in life. Please note that in some cases there is a possibility to reveal also the risk for one of the parents, who may not know or wish to know their status. And in this case, prenatal testing must be preceded by genetic counseling.

For autosomal recessive neuromuscular disorders, such as Friedreich's ataxia and spinal muscular atrophy, molecular genetic testing is applied:

- as a diagnostic test in patients manifesting clinical symptoms, in order to confirm the exact type of disorder and reveal the precise genetic lesion
- for carrier detection in the population or in members of a family with affected relatives, prior to pregnancy or very early in pregnancy
- in prenatal diagnosis, in cases where both parents are known carriers and the genetic lesion in both is already known (a necessary prerequisite), in order to determine whether the fetus is at risk of being affected later in life.

Finally, for the sex-linked neuromuscular diseases, such a muscular dystrophy Duchenne/Becker, Charcot-Marie-Tooth CMTX1, Kennedy's disease and Rett syndrome, molecular genetic testing is applied:

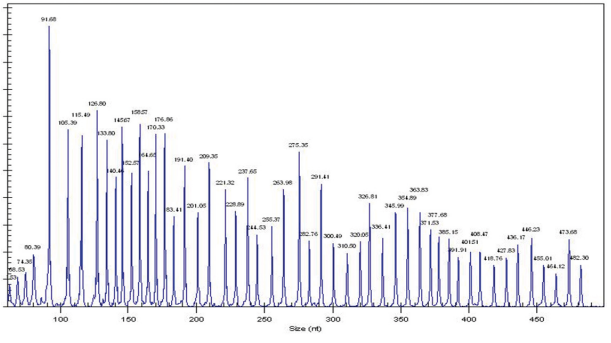
- in affected males or females (depending) as a diagnostic test, in order to confirm the exact type of disorder and reveal the precise genetic lesion

- to identify female carriers, who are members of a family with affected relatives (e.g. affected brother), prior to pregnancy or early in pregnancy

- in prenatal diagnosis, in cases where the mother is a known carrier and the genetic lesion is already known (a necessary prerequisite), in order to determine whether the fetus is at risk of being affected later in life

NOTE: Our laboratory participates successfully in the external quality control organized by the European Molecular Genetics Quality Network (EMQN), which is applied periodically for many of the above neurodegenerative diseases.

Our participations ensures the highest possible diagnostic accuracy of our tests and affords confidence to our results.



InterGenetics
DIAGNOSTIC GENETIC CENTER

120, Vas. Sofias Av.,
11526 Athens, Greece
T/ (+30) 2107705010 • 2107756588
T/ (+30) 2107705125 • 2104177919
F/ (+30) 2107705011

info@intergenetics.eu
www.intergenetics.eu