International support: Molecular diagnoses free of charge for five rare diseases

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INTRODUCTION

The B.I.R.D. foundation is committed to rare diseases and particular attention is given to spreading the awareness of rare diseases. With this in mind the foundation with its supporters started performing free diagnostic testes for the countries where such procedures were not yet available.
Initially free diagnostic tests were available only for Prader – Willi syndrome and conducted in association with the International Prader – Willi Syndrome Organisation (IPWSO). The very positive response obtained induced the foundation to search for new partners to extend such service to other diseases.
Currently the foundation can offer free of charge diagnostic testing for five rare diseases: Prader – Willi syndrome, APECED, Lesch – Nyhan syndrome, Krabbe disease and Metachromatic Leukodystrophy. The tests are performed on gDNA extracted from dried blood spots (DBS) on filter paper. With the exception of Prader - Willi syndrome, where the test consists of a MS-PCR, all the other tests consist in direct gDNA sequencing. All analyzes are performed according to current EU guidelines maintaining very strict laboratory standards.
Special care was given to cost containment for the referring physician selecting a widely available sampling method (DBS on filter paper) and maintaining the sample sending procedure as simple as possible (regular letter).
Up to today, more than 250 samples have been analyzed from 25 countries from Asia, South America, Europe and Africa.
Particular care was given to the procedure required to send samples for analysis that takes into account possible delays in the delivery, ease of collecting the biological sample and not last the cost. The optimal solution determined was performing the tests on DNA extracted from dried blood spots on filter paper (detailed instructions for sending a sample for analysis can be obtained contacting the author). The advantage of dried blood spots is they maintain DNA stable at room temperature, are very easy to prepare and can be sent via regular mail, significantly lowering costs.

PRADER – WILLI SYNDROME

The early diagnosis of Prader Willi syndrome (PWS) is essential for the treatment of the patients and has a dramatic impact on both quality of life and life expectancy. There are two main conditions necessary to guarantee an early diagnosis: knowledge of PWS with clear diagnostic guidelines and the availability of a molecular test to confirm the clinical suspect.
Since 2004 the "Mauro Baschirotto" Institute for Rare Diseases (BIRD) together with the International Prader-Willi Syndrome Organisation (IPWSO) are offering free diagnostic tests for PWS for the countries where such service is not yet available (Figure 1). This experimental program has been a great aid for physicians in confirming the diagnosis of PWS.
The testing method we use, called methylation specific PCR or MS-PCR, has a sensitivity of 99% in detecting PWS (Table 1) and offers a very cost effective way of analyzing the samples. This test is conducted by a modification of the DNA using the sodium salt of the bisulphite ion (HSO3-). This modification is sensible to methylation and only non methylated DNA is altered. Using specific probes for the modified DNA we are able to determine the paternal or maternal pattern of methylation in the SNRPN region analyzed. The fragments corresponding to the maternal and paternal imprint are of different sizes, making it easy recognizing them by a simple electrophoresis on agarose gel (Figure 2).

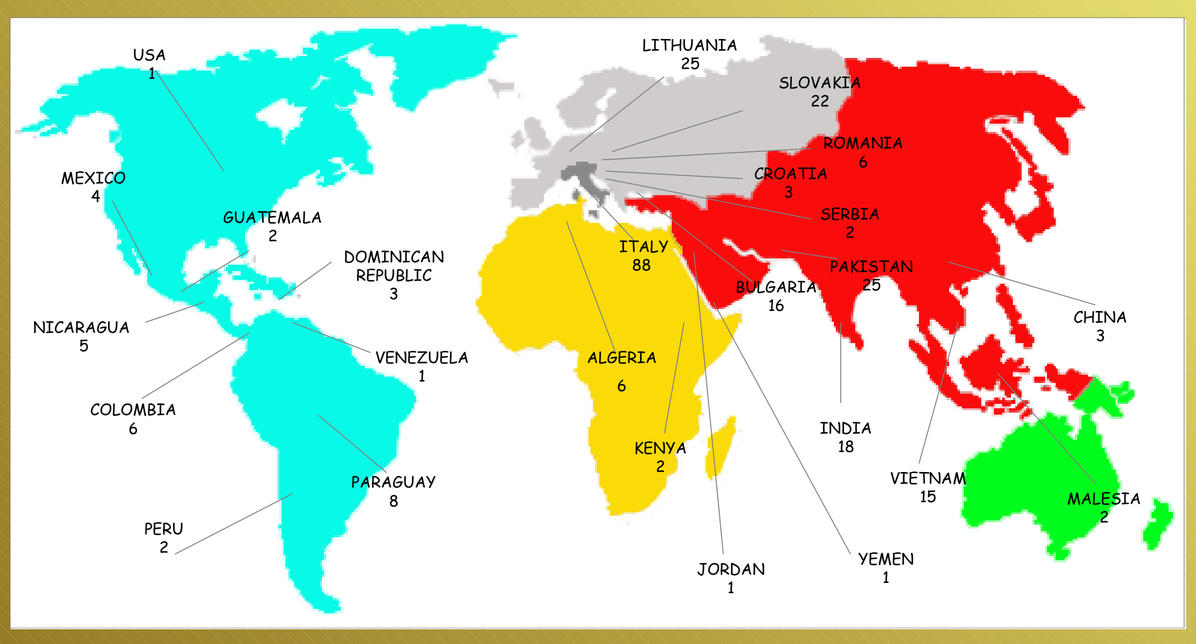

Figure 1: Diagnoses 2003-2009: 270 PWS from all over the world (91 Italians and 179 from abroad)

Figure 2: the lanes from left to right are DNA ladder, sample 1-6, Positive Control, Negative Control, non methylated DNA, reagent check, DNA ladder. As we can see negative samples show both the paternal and maternal pattern of methylation with two visible bands, lanes 2-3 and 9, the Prader Willi samples show only the presence of maternal pattern of methylation, lanes 6-7 and 8, while Angelman Syndrome show only the paternal pattern of methylation, lanes 4-5. (The same test can also identify around 70% of Angelman Syndrome (AS) cases as the gene responsible for AS is present in the same region.)

LESCH – NYHAN SYNDROME

Lesch-Nyhan syndrome (LNS) is a rare, inherited disorder caused by a deficiency of the enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT). LNS is an X-linked recessive disease-- the gene is carried by the mother and passed on to her son. LNS is present at birth in baby boys. The lack of HPRT causes a build-up of uric acid in all body fluids, and leads to symptoms such as severe gout, poor muscle control, and moderate retardation, which appear in the first year of life. A striking feature of LNS is self-mutilating behaviors – characterized by lip and finger biting – that begin in the second year of life. Abnormally high uric acid levels can cause sodium urate crystals to form in the joints, kidneys, central nervous system, and other tissues of the body, leading to gout-like swelling in the joints and severe kidney problems. Neurological symptoms include facial grimacing, involuntary writhing, and choreic movements.

The diagnosis of LNS is confirmed by molecular genetic testing of the HPRT1 gene by amplification and direct sequencing of all the 9 coding exons. The testing method is expected to detect 95% of HPRT1 mutations. Small deletions, insertions, and inversions within exons can be easily seen on genomic sequencing.

KRABBE LEUKODYSTROPHY

Krabbé disease is a rare, inherited degenerative disorder of the central and peripheral nervous systems. It is characterized by the presence of globoid cells (cells that have more than one nucleus), the breakdown of the nerve's protective myelin coating, and destruction of brain cells.

Krabbé disease is caused by a deficiency of galactocerebrosidase, an essential enzyme for myelin metabolism. The disease most often affects infants, with onset before age 6 months, but can occur in adolescence or adulthood. Symptoms include irritability, unexplained fever, limb stiffness, seizures, feeding difficulties, vomiting, and slowing of mental and motor development. Other symptoms include muscle weakness, spasticity, deafness, and blindness.

The diagnosis of Krabbé disease is confirmed by molecular genetic testing of the GALC gene by amplification and direct sequencing of all the 17 exons. The testing method is expected to detect more than 99% of GALC mutations. Small deletions, insertions, and inversions within exons can be easily seen on genomic sequencing.

METACHROMATIC LEUKODYSTROPHY

Metachromatic leukodystrophy (MLD) is one of a group of genetic disorders called the leukodystrophies. These diseases impair the growth or development of the myelin sheath, the fatty covering that acts as an insulator around nerve fibers. MLD is caused by a deficiency of the enzyme arylsulfatase A. MLD is one of several lipid storage diseases, which result in the toxic buildup of fatty materials (lipids) in cells in the nervous system, liver, and kidneys. There are three forms of MLD: late infantile, juvenile, and adult. In the late infantile form, which is the most common MLD, affected children have difficulty walking after the first year of life. Symptoms include muscle wasting and weakness, muscle rigidity, developmental delays, progressive loss of vision leading to blindness, convulsions, impaired swallowing, paralysis, and dementia. Children may become comatose. Most children with this form of MLD die by age 5. Children with the juvenile form of MLD (between 3-10 years of age) usually begin with impaired school performance, mental deterioration, and dementia and then develop symptoms similar to the infantile form but with slower progression. The adult form commonly begins after age 16 as a psychiatric disorder or progressive dementia. Adult-onset MLD progresses more slowly than the infantile form.

The diagnosis of MLD is confirmed by molecular genetic testing of the ARSA gene by amplification and direct sequencing of all the 9 coding exons and of the PSAP gene by amplification and direct sequencing of all the 14 coding exons. The testing method is expected to detect 97% of ARSA mutations and more than 90% of PSAP mutations. Small deletions, insertions, and inversions within exons can be easily seen on genomic sequencing.

AUTOIMMUNE POLIENDOCRINE SYNDROME TYPE 1

Autoimmune polyendocrine syndrome type 1 (APS1) is a very rare genetic syndrome involving in the autoimmune system. It is a combination of several distinct disorders and is defined as the subnormal functioning of several endocrine glands at the same time (concurrently). The old name for the condition was APECED, an acronym that stands for Autoimmune Polyendocrinopathy (APE), Candidiasis (C) and Ectodermal Dysplasia (ED). Autoimmune disease affecting one gland is frequently followed by the impairment of other glands. In its classic form it affects children and adults younger than age 35. It is characterized by below normal secretion of the parathyroid gland (hypoparathyroidism 79%) and the failure of the adrenal cortex to secrete normal volumes of hormones (72%). About 60% of women and about 15% of men fail to mature sexually (hypogonadism). A persistant fungal infection (mucocutaneous candidiasisis) is common and chronic.

The diagnosis of APS1 is confirmed by molecular genetic testing of the AIRE gene by amplification and direct sequencing of all the 14 coding exons. The testing method is expected to detect more than 80% of AIRE mutations. Small deletions, insertions, and inversions within exons can be easily seen on genomic sequencing.