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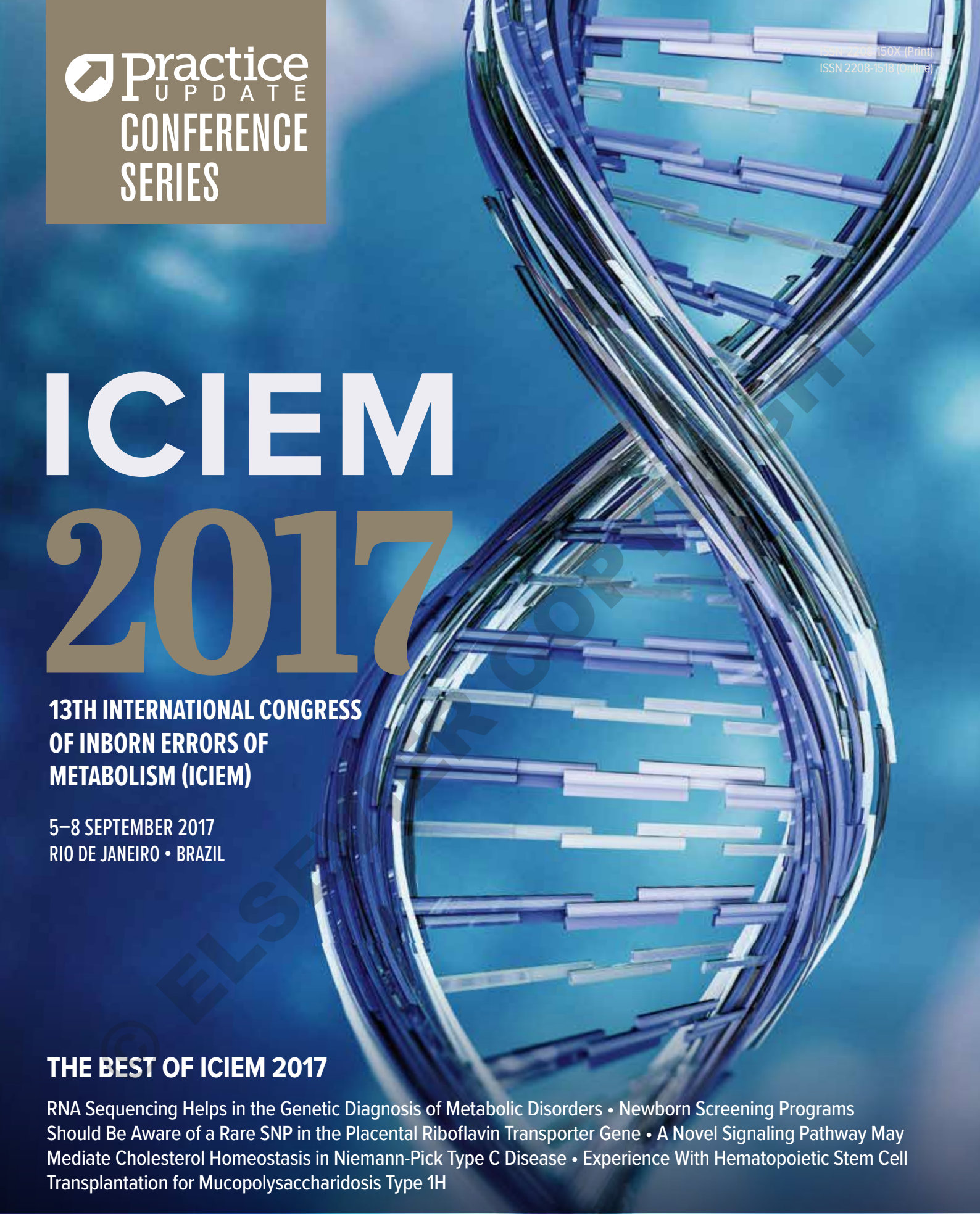

ICIEM 2017

13TH INTERNATIONAL CONGRESS OF INBORN ERRORS OF METABOLISM (ICIEM)

5–8 SEPTEMBER 2017
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THE BEST OF ICIEM 2017

RNA Sequencing Helps in the Genetic Diagnosis of Metabolic Disorders • Newborn Screening Programs Should Be Aware of a Rare SNP in the Placental Riboflavin Transporter Gene • A Novel Signaling Pathway May Mediate Cholesterol Homeostasis in Niemann-Pick Type C Disease • Experience With Hematopoietic Stem Cell Transplantation for Mucopolysaccharidosis Type 1H

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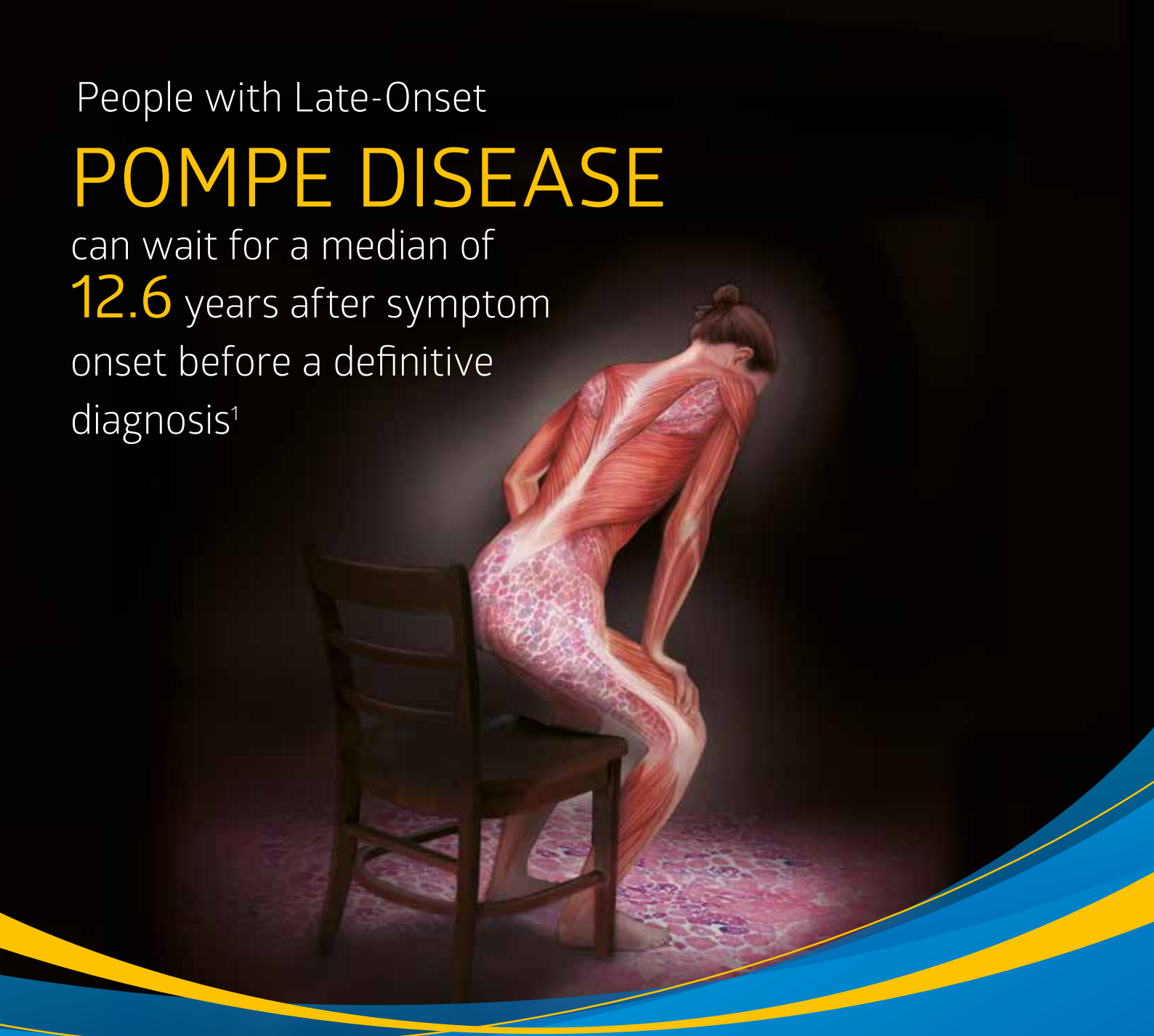
People with Late-Onset

POMPE DISEASE

can wait for a median of **12.6** years after symptom onset before a definitive diagnosis[1]



Don't delay the diagnosis of this rare & life-threatening condition that can affect both juveniles and adults[1]

Test promptly for Late-Onset Pompe disease in patients with proximal muscle weakness, respiratory insufficiency, unclassified limb-girdle muscular dystrophy, or asymptomatic hyperCKemia with unknown cause.[2,3]

Email DBSkits@sanofi.com to request your free dried blood spot (DBS) kits to screen and test for Pompe disease.

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References: 1. Kishnani PS *et al. Am J Med Genet A.* 2013; 161: 2431–43. **2.** Kishnani PS *et al. Genet Med* 2006; 8: 267–88. **3.** Musumeci O *et al. J Neurol Neurosurg Psychiatry* 2016; 87: 5–11. Sanofi-aventis Australia Pty Ltd trading as Sanofi Genzyme ABN 31 008 558 807. Talavera Corporate Centre, Building D, 12–24 Talavera Road, Macquarie Park, NSW 2113. GZANZ.MYOZ.17.04.0053a. August 2017. AM7073.

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RNA Sequencing Helps in the Genetic Diagnosis of Metabolic Disorders

RNA sequencing in combination with bioinformatics-filtering criteria helps bridge the gap in patients suspected of suffering from a metabolic disorder who remain undiagnosed after whole exome sequencing.

Dr. Holger Prokisch

Holger Prokisch, MD, and Laura Kremer, MS, of the Helmholtz Zentrum München, Neuherberg, Germany, explained that in recent years, whole exome sequencing has become the gold standard for molecular diagnosis. As many as half of patients across a variety of metabolic disorders, however, do not receive a diagnosis by whole exome sequencing.

Dr. Prokisch and Ms. Kremer reasoned that inconclusive whole exome sequencing can be attributed to incomplete capture of variants, especially noncoding variants, or failure to prioritize them. The former can be overcome by whole genome sequencing.

The vast number of variants generated by whole genome sequencing and poor understanding of the noncoding genome, however, obscure prioritization. RNA sequencing may ease prioritization of variants by unraveling their effects on RNA abundance and sequence.

Dr. Prokisch said: "Taken in the aggregate, so-called rare illnesses are anything but rare. They affect about 8% of the global population. The majority of these conditions have genetic causes. It is important to determine which genes trigger an illness when developing a treatment.

> "Exome-wide sequencing has revolutionized molecular diagnostics in patients with suspected inborn errors of metabolism.

"Exome-wide sequencing has revolutionized molecular diagnostics in patients with suspected inborn errors of metabolism. Compared to the pre-exome sequencing era, the diagnostic yield of up to 60 % in mitochondrial disorders, for example, is impressive.

"A large fraction of individuals," he said, "is left without a diagnosis, however. The gap in diagnostic yield indicates a causative role of variants not covered by exome sequencing, for example, nonexonic regulatory variants.

"Assuming this shortcoming," he added, "we started to search for noncoding variants by focusing on ribonucleic acid (RNA). RNA is the name of a group of cellular molecules whose function includes executing blueprints coded in DNA. Based on the composition and number of RNA molecules, we can draw conclusions about specific problems in executing the DNA code."

The investigators performed RNA sequencing on 105 fibroblast cell lines from patients with a suspected metabolic disorder, including 48 patients in whom whole exome sequencing had been inconclusive.

To estimate their association with disease, the team systematically prioritized genes with aberrant expression level, aberrant splicing, and monoallelic expression of rare variants. The analysis identified per sample an average of six monoallelic-expressed variants, one expression outlier, and approximately five splice defects. This small number of events allowed manual inspection and validation.

Follow-up studies in two patients with respiratory chain complex I deficiency yielded an expression outlier in the respiratory chain complex I assembly factor translocase of inner mitochondrial membrane domain containing 1 (TIMMDC1), a gene not annotated previously with disease risk.

The investigators subsequently identified a deep intronic variant, probably activating a cryptic splice site that resulted in aberrant splicing. They confirmed the causal role of TIMMDC1 deficiency. In additional patients, they further identified RNA effects of variants of unknown significance in CLPP, MCOLN1, and ALDH18A1 and were able to subsequently establish their pathogenicity.

Surprisingly, they also found that synonymous variants in TAZ and GAMT, respectively, caused pathogenic splice defects in two cases. In total, they provided a genetic diagnosis for 15% of unsolved whole exome sequencing cases. Further validation of strong candidates in additional samples is ongoing.

Dr. Prokisch concluded that RNA sequencing in combination with bioinformatics-filtering criteria helps bridge the gap in patients suspected of suffering from a metabolic disorder who remain undiagnosed after whole exome sequencing. Importantly, this approach applies to any rare disease setting and allows for discovery of new disease-associated genes.

Dr. Prokisch predicted that RNA sequencing will become essential in genome sequencing. "With increasing genome-wide molecular diagnostics," he said, "RNA sequencing will be needed to interpret noncoding variants. It will be implemented in future diagnostic processes to maximize the diagnostic yield."

He added, "Variation in the noncoding region of the genome may contribute more to Mendelian disorders than thought to date. We are moving the pipeline from research to diagnostics. Physicians need to be trained to consider early on in the diagnostic path, when they take samples from the patient to consider an extra sample for the RNA analysis."

"Patients without a diagnosis despite genome-wide sequencing are now investigated for pathogenic variants that affect execution of the blueprint." ■

www.practiceupdate.com/c/59037

UPLC-MS/MS Oligosaccharide Analysis Improves the Diagnosis and Monitoring of Patients With Glycoprotein Storage Disorders

Ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) oligosaccharide analysis has been shown to improve the diagnosis and monitoring of patients with glycoprotein storage disorders, results of a validation study suggest.

Glycoprotein storage disorders are a subset of the larger lysosomal storage disease group, which consist of over 50 autosomal recessive inherited metabolic diseases.

Glycoprotein storage disorders affect multiple body systems. Clinical symptoms may vary from patient to patient, and even among siblings. For most children, the implications are eventual loss of mental and physical functions, and a premature death.

The glycoproteinoses are characterized by the accumulation of disease-specific oligosaccharides. Glycoproteinoses result from defects in lysosomal function. The term is sometimes reserved for conditions involving degradation of glycoproteins.

According to a Canadian study, approximately 2.3 children per 100,000 births (one in 43,000) suffer from some form of glycogen storage disease. In the US, they are estimated to occur in one per 20,000–25,000 births. A Dutch study estimated an incidence of one in 40,000.

Treatment is typically with frequent small meals of carbohydrates and cornstarch to prevent low blood sugar. Other treatments may include allopurinol, human granulocyte colony stimulating factor, recombinant human α-mannosidase, and recombinant human aspartylglucosaminase.

Taraka R. Donti, PhD, of the Greenwood Genetic Center, South Carolina, explained that the majority of clinical laboratories utilize thin-layer chromatography to measure urinary free oligosaccharides for identification of patients with a variety of inborn errors of metabolism, including glycoprotein storage disorders, Pompe disease, and more recently, several congenital disorders of glycosylation.

Thin-layer chromatography is not an optimal assay, however, as it is not quantitative and lacks the sensitivity and specificity of a clinical diagnostic test.

Dr. Donti and colleagues developed a novel, rapid UPLC-MS/MS method to measure urinary free oligosaccharides using reducing-end labeling. The relative concentration of nine disease-specific oligosaccharides is determined by comparison vs the peak area of a single internal standard.

The investigators analyzed 51 urine samples from a patient cohort encompassing eight diseases:

- Aspartylglucosaminuria
- Fucosidosis
- a-mannosidosis
- b-mannosidosis
- b-galactosidase deficiency
- Sandhoff disease
- Sialidosis
- Galactosialidosis

Samples were collected as part of the Glycoproteinoses Natural History Study or through routine diagnostic testing. Age-specific normal ranges were developed using 110 samples from unaffected controls.

Increased abundance of the disease-specific oligosaccharide was identified in all 51 affected individuals. Compared with age-matched controls, elevations ranged from 5- to 2100-fold, with fucosidosis (1285-fold), sialidosis (426-fold), galactosialidosis (265-fold), and aspartylglucosaminuria (154-fold) exhibiting the widest dynamic range.

Urine samples from patients with α-mannosidosis, fucosidosis, and β-mannosidosis post bone marrow transplantation exhibited significantly lower oligosaccharide levels than untreated patients. This indicated that the assay can be used to evaluate the efficacy of future treatments.

The team also analyzed 80 urine samples from patients with mucolipidosis types II, II/III, or III, and identified at least one free oligosaccharide abnormality in all mucolipidosis patients. The team was also capable of differentiating between patients with mucolipidosis II vs III.

Identification of significant elevations in urinary free oligosaccharides specific for Pompe disease (Glc4) and two types of congenital disorders of glycosylation suggested that the assay can be used as a broad screen for an increasing number of inborn errors of metabolism.

> "Based on data accumulated to date, the assay is a significant improvement over thin-layer chromatography and capable of avoiding false-positives caused by dietary or medication-related metabolites.

Dr. Donti concluded that, based on data accumulated to date, the assay is a significant improvement over thin-layer chromatography and capable of avoiding false-positives caused by dietary or medication-related metabolites.

The assay provides a sensitive method to diagnose patients with lysosomal diseases and could replace thin-layer chromatography. It utilizes a triple quadrupole tandem mass spectrometer rather than a matrix-assisted laser desorption/ionization (MALDI) time of flight (TOF) instrument, which renders the assay applicable to more clinical laboratories.

The assay can be used to evaluate the efficacy of future treatments. Preliminary results indicate that the assay can be used in other specimen types such as dried blood spots, plasma, leukocytes, and fibroblasts, broadening its clinical utility. Finally, for improved accuracy and reproducibility, absolute quantification can be achieved using oligosaccharide-specific standards and internal standards. ■

www.practiceupdate.com/c/59032

Newborn Screening Programs Should Be Aware of a Rare SNP in the Placental Riboflavin Transporter Gene

Newborn screening programs should be aware of a rare single-nucleotide polymorphism in the placental riboflavin transporter gene that causes transient multiple Acyl-CoA dehydrogenation deficiency (MADD).

Rikke Katrine Jentoft Olsen, MD, of Aarhus University, Denmark, explained that MADD is a rare inborn error of metabolism that may result in a favorable outcome when treated with high doses of riboflavin.

Patients with MADD fall into three broad clinical phenotypes:

1. Neonatal onset with congenital anomalies. Affected neonates are often premature, presenting with severe nonketotic hypoglycemia, hypotonia, hepatomegaly and severe metabolic acidosis within the first 24 h of life. They usually harbor dysplastic kidneys with multiple cysts and may also exhibit facial dysmorphism (low-set ears, high forehead, hypertelorism, and hypoplastic midface); rocker-bottom feet; and anomalies of external genitalia. Death usually occurs within the first week of life.
2. Neonatal onset without anomalies (together called MADD-severe). These patients usually present within the first 24–48 h of life with hypotonia, tachypnea, hepatomegaly, metabolic acidosis, and hypoketotic hypoglycemia. Most die during the first week(s) of life but some have survived for several months, usually dying of severe cardiomyopathy.
3. Mild and/or late-onset (MADD-mild). MADD-mild patients show a broad clinical spectrum of disease. Onset of intermittent episodes of vomiting, metabolic acidosis, and hypoketotic hypoglycemia (with or without cardiac involvement) can occur during the first few months of life up to adolescent/adult presentation with acute Reye-like illness with ketoacidosis and lipid storage myopathy. The latter often respond to pharmacological doses of riboflavin.

The birth prevalence of MADD is estimated at one in 200,000 individuals, but great variation is seen between countries/ethnicities. The incidence appears to be considerably rarer in Asian populations.

Though many individuals who harbor a defective AMPD gene are asymptomatic, others may suffer from symptoms such as exercise intolerance, muscle pain, and muscle cramping.

It is important for patients with MADD to maintain strength and fitness without exercising or working to exhaustion. Learning this balance can be difficult.

Symptomatic relief of the effects of MADD can be achieved by administering oral ribose 10 g per 100 pounds (0.2 g per kilogram) of body weight per day, and exercise modulation as appropriate.

Taken hourly, ribose provides a direct but limited source of cellular energy. Patients with myoadenylate deaminase deficiency do not retain ribose during heavy exercise, so supplementation may be required to rebuild levels of adenosine triphosphate.

Creatine monohydrate may also be helpful, as it provides an alternative source of energy for anaerobic muscle tissue and was found to be helpful for other, unrelated muscular myopathies.

Potential complications of MADD include:

- Increased risk that a statin will cause myopathy
- Malignant hyperthermia from anesthesia, with permanent muscle damage. Patients with MADD

are advised to notify their anesthesiologist about their condition prior to surgery

In most cases where myopathy is present with MADD, a second muscle disease is present and symptoms are worse than either disease in isolation.

MADD is most often caused by recessive mutations in genes coding for electron transfer flavoprotein and its dehydrogenase, which link mitochondrial flavin adenine dinucleotide (FAD)-containing acyl-CoA dehydrogenation reactions to adenosine triphosphate (ATP) production in the respiratory chain.

More recently, MADD has been linked to mutations in genes involved in cellular riboflavin transport or in synthesis of the FAD cofactor from riboflavin. Fetal riboflavin status depends largely on the availability of riboflavin in maternal circulation and placental transport of riboflavin.

Thus, maternal riboflavin deficiency and/or gene defects in placental riboflavin transport can potentially cause transient MADD and significant disease in the newborn.

A single case of transient MADD has been reported in the child of a mother who carried a heterozygous deletion of the SLC52A1 gene responsible for placental riboflavin transport.

> "The c.1134t11G>A mutation carries a minor allele frequency of 0.2% in the general population and could be a risk factor for development of transient MADD and significant illness in children of pregnant mothers with subclinical riboflavin deficiency.

Dr. Jentoft Olsen and colleagues reported another case of transient MADD, caused by a rare single-nucleotide polymorphism in SLC52A1.

The newborn girl presented in the first few days of life with hypotonia, lethargy, and metabolic lactic acidosis. Newborn screening filter card analysis revealed elevated multiple acyl-carnitines (C6-C14), resembling the MADD profile.

MADD biochemistry was confirmed by analysis of plasma acylcarnitines and urine organic acids. Riboflavin treatment corrected the MADD biochemistry and clinical symptoms. Analysis of the mother's riboflavin status showed that she was borderline riboflavin deficient.

Sequencing of MADD candidate genes revealed that mother and daughter were carriers of a c.1134þ11G>A mutation in SLC52A1. Using splicing reporter minigenes and RNA affinity purification of nuclear splice proteins, the mutation creates a binding site for the splice-inhibitory hnRNP A1 protein and causes exon 4 skipping.

Dr. Jentoft Olsen concluded that the c.1134þ11G>A mutation carries a minor allele frequency of 0.2% in the general population and could be a risk factor for development of transient MADD and significant illness in children of pregnant mothers with subclinical riboflavin deficiency.

Newborn screening programs should be aware of this MADD-associated single nucleotide polymorphism. ■

www.practiceupdate.com/c/59036

Four Novel α-Galactosidase A Gene Mutations are Identified in Peruvian Families with Fabry Disease

Four novel α-galactosidase A gene mutations have been identified in Peruvian families with Fabry disease.

Gioconda Carmen Elena Manassero Morales, MD, of the National Institute of Child Health, San Borja, Lima, Peru, explained that mutations in the a-galactosidase A gene lead to Fabry disease, an X-chromosomal inherited lysosomal storage disorder of glycosphingolipids produced by a deficit of lysosomal enzyme a-galactosidase A.

The disease causes lipid accumulation in the central nervous system, heart, kidneys, and skin. This accumulation can lead to pain, kidney failure, heart disease, and stroke. Symptoms begin at an early age. All Fabry disease is progressive and may lead to organ damage regardless of age at symptom onset.

Cardiac complications such as heart failure and myocardial infarction are the main cause of death in patients with Fabry disease.

The estimated incidence of Fabry disease is one in 50,000 males worldwide. An estimated 3000 individuals in the US have been diagnosed with Fabry disease, more than any other country. The incidence in Peru has not been established.

Life expectancy of males with Fabry disease is 58.2 years, vs 74.7 years in the general population. That of affected females is 75.4 years vs 80.0 years in the general population, according to registry data from 2001 to 2008.

Fabry disease is suspected based on the individual's clinical presentation, and can be diagnosed by an enzyme assay (usually done on leukocytes to measure the level of a-galactosidase activity. An enzyme assay is not reliable for the diagnosis of disease in females due to the random nature of X-inactivation.

The X-linked recessive DNA mutations that cause the disease exhibit incomplete penetrance in heterozygous females. The condition affects hemizygous males (that is, all males), as well as homozygous, and in many cases, heterozygous females.

While males typically experience severe symptoms, women can range from being asymptomatic to suffering from severe symptoms. New research suggests many women suffer from severe symptoms ranging from early cataracts or strokes to hypertrophic left ventricular heart problems and renal failure. This variability is thought to be due to X-inactivation patterns during embryonic development of the female.

Molecular genetic analysis of the GLA gene is the most accurate method of diagnosis in females, particularly if mutations have been identified in male family members. Many disease-causing mutations

Lipidomics are a New Tool to Identify Unrecognized Defects in Fatty Acid Homeostasis

Lipidomics have been described as a new tool for identifying unrecognized defects in fatty acid homeostasis.

Benoit Colsch, MD, of the Alternative Energies and Atomic Energy Commission (CEA), Gif-Sur-Yvette, France, described the emergence of untargeted lipidomic approaches to the understanding of lipid pathways at ICIEM 2017. He noted that lipids are essential to the integrity of cell membranes. Lipids also perform many biological functions linked to energy storage and cell signaling. They are involved in a large number of heterogeneous diseases such as cancer, diabetes, neurological disorders, and inherited metabolic diseases.

Lipidomic profiles of human biological materials for biomarker discovery are mostly performed in plasma, cell, or tissue extracts, and to a lesser extent, in urine.

Due to high structural diversity of the lipidome, simultaneous detection of minor and major lipid species using mass spectrometry remains a challenge. Multiple isobaric and isomeric lipid species, in addition to numerous distinct lipid classes, add to the challenge of characterizing the lipidome in complex biological matrices.

The first analysis of complex lipid mixtures by mass spectrometry was introduced in the 1990s by Han and Gross. Since then, improvements in mass spectrometry instrumentation in terms of mass resolution, mass accuracy, and duty cycles have expanded research in lipidomics.

In targeted approaches, the overall platform, that is, sample preparation and mass spectrometry detection is optimized for a predetermined number of lipid classes or sub-classes.

These methods are based mainly on low-resolution, triple quadrupole, precursor-ion scanning, neutral loss scanning, and product ion modes. They offer high sensitivity and have been applied successfully to lipid profiling of various biomaterials.

A remarkable contribution in this field was the work of Quehenberger et al who quantified over 500 distinct molecular species distributed among the main lipid categories in plasma samples in 2010. Numerous targeted methods were necessary to achieve this broad lipidome coverage, however, limiting the throughput capabilities of such approaches.

have been noted. Kidney biopsy may also suggest Fabry disease if excessive lipid buildup is noted. Pediatricians, as well as internists, commonly misdiagnose Fabry disease.

The Human Gene Mutation Database, Fabry mutation database, and Clin Var database contain hundreds of registered mutations of α-galactosidase A gene-encoding α-galactosidase A.

Dr. Manassero Morales and Kelly Cinthia Franco Bustamante, MD, also of the National Institute of Child Health, reported four novel mutations in the α-galactosidase A gene and characterized 14 Peruvian families with Fabry disease molecularly.

A screening program using α-galactosidase A activity in blood was performed in patients undergoing hemodialysis at the largest hospitals in Peru. Complete sequencing of the α-galactosidase A gene was performed in those with confirmed reduced enzymatic activity in leukocytes

A family tree was constructed for each proband, including all members of at least four generations. Enzymatic testing and testing targeting molecular familial mutations were performed in all available at-risk family members.

After screening, 16 patients presented reduced enzyme activity confirmed in leukocytes. A total of 13 different mutant alleles were identified in these families; three mutations (p.D109G, p.K130T, and p.R363H) were found to be shared by 7 families.

Four novel missense mutations were detected (p.G35A, p.I64F, p.K130T, and p.G171S). One of these mutations was shared by two families. Family trees were constructed for 14 families with 1674 members, of whom 446 members identified as subjects at risk of carrying a mutation were studied to locate their targeted familial mutation.

One-third (n=147) were carriers of their specific familial mutation. Of these, 52 (34%) heterozygous males and 95 (66%) hemizygous females were found. A total of 22 male patients and one symptomatic woman are undergoing enzyme replacement therapy.

Dr. Manassero Morales concluded that complete molecular analysis of the α-galactosidase A gene performed in 16 Peruvian families showed 13 different genotypes. Four novel missense mutations were found.

Identification of the familial α-galactosidase A gene mutation enabled targeted investigation in at-risk family members with the goal of identifying symptomatic patients and recommending early enzymatic replacement treatment. ■

www.practiceupdate.com/c/59035

By enabling accurate mass measurements with sub-ppm errors, high resolution mass spectrometers, which include Fourier transform ion cyclotron resonance, Orbitrap, and time-of-flight instruments, have prompted the use of untargeted lipidomic approaches by affording the possibility to separate isobaric lipid species.

Using high-resolution mass spectrometry instruments, "shotgun approaches" have emerged. These are based on direct introduction of a total lipid extract into the mass spectrometer. They were developed for global lipidomic analysis and enable the measurement of several hundred of lipid species covering the 21 major lipid classes from yeast extracts.

Though these methods, without prior chromatographic separation, are fast and simple, however, their sensitivity is limited by major ion suppression effects and the lack of discrimination between isomeric lipid species.

Other tactics include hyphenated methods such as liquid chromatography and supercritical fluid chromatography.

Along with the evolution in instrumentation in mass spectrometry, bioinformatic tools have been developed in the field of lipidomics to handle, process, and interpret large amounts of data.

Before automatic detection and annotation, raw data must be converted into data formats compatible with peak detection and alignment software tools such as MZmine or XCMS.

Thanks to accurate mass measurements provided by high-resolution mass spectrometry, lipidomic features can be annotated using lipid databases such as that of the LIPID MAPS consortium, which introduced the Comprehensive Classification System for Lipids. This classification system aims to catalog lipid species and makes available online tools to support lipid identifications.

More recently, the LipidBlast in silico tandem mass spectral database has been implemented and covers compounds of 26 lipid classes. In parallel, manufacturers have also developed commercial software tools, such as Lipid Search, Lipid View, and SimLipid, for direct annotation from raw data.

Reliable lipidomic data treatment workflows able to handle the detection and alignment of features, however, together with selection and annotation of analytically reliable ones, are still emerging.

With the emergence of high-resolution mass spectrometry and the capability of instrumentation to perform simultaneous analyses (mass spectrometry and mass spectrometry/mass spectrometry experiments), the major challenge of using untargeted lipidomic approaches is to deal with the vast amount of information generated by data acquisition and databases available for lipid annotation.

The ultimate goal is to better understand lipid pathways impacted by various diseases. ■

www.practiceupdate.com/c/59031

A Novel Signaling Pathway May Mediate Cholesterol Homeostasis in Niemann-Pick Type C Disease

A novel signaling pathway involving two proteins may modulate cholesterol homeostasis in Niemann-Pick type C disease, according to results of an in vitro and murine analysis, presented at ICIEM 2017.

Niemann-Pick type C is a lysosomal storage disease associated with mutations in the NPC1 and NPC2 genes. Niemann-Pick type C affects an estimated one in 150,000 individuals. Approximately 50% of cases present before 10 years of age, but manifestations may be first recognized as late as the sixth decade of life.

Niemann-Pick type C presents in a wide clinical spectrum. Affected individuals may exhibit enlargement of the spleen and liver, or enlarged spleen or liver combined, yet this finding may be absent in later-onset cases. Prolonged jaundice or elevated bilirubin can present at birth. In some cases, however, enlargement of the spleen or liver does not occur for months or years, or not at all.

Enlargement of the spleen or liver frequently becomes less apparent with time, in contrast to the progression of other lysosomal storage diseases such as Niemann-Pick disease types A and B or Gaucher disease. Organ enlargement does not usually cause major complications.

Progressive neurological disease is the hallmark of Niemann-Pick type C disease, and is responsible for disability and premature death in all cases beyond early childhood. Classically, children with Niemann-Pick type C may present initially with a delay in reaching normal developmental milestone skills before manifesting cognitive decline.

Neurological signs and symptoms include cerebellar ataxia, dysarthria, dysphagia, tremor, partial or generalized epilepsy, vertical supranuclear palsy, sleep inversion, gelastic cataplexy, dystonia, spasticity, hypotonia, ptosis, microcephaly, psychosis, progressive dementia, progressive hearing loss, bipolar disorder, major and psychotic depression that may include hallucinations, delusions, mutism or stupor.

In the terminal stages of Niemann-Pick type C disease, the patient is bedridden, with complete ophthlamoplegia, loss of volitional movement, and severe dementia.

> "The results strongly suggest that transcription factor EB tyrosine phosphorylation by c-Abl impacts transcription factor EB nuclear translocation. This phosphorylation and resulting translocation suggests a novel signaling pathway involving these two proteins.

Niemann-Pick type C is biochemically, genetically, and clinically distinct from Niemann-Pick types A and B. In types A and B, the lysosomal enzyme acid sphingomyelinase is completely or partially deficient.

In Niemann-Pick type C, the protein product of NPC1, the major mutated gene, is not an enzyme but appears to function as a transporter in the endosomal-lysosomal system. This transporter moves large water-insoluble molecules through the cell.

The protein coded by the NPC2 gene structurally resembles an enzyme more closely but seems to act in cooperation with the NPC1 protein in transporting cellular molecules. Disruption of this transport system results in the accumulation of cholesterol and glycolipids in lysosomes.

Silvana Zanlungo, MD, of the Pontificia Universidad Católica de Chile, Santiago, explained that transcription factor EB is the master regulator of the lysosome biogenesis and function, as well as the autophagy pathway.

Activity and translocation to the nucleus of transcription factor EB depends on its phosphorylation state. Inhibition of the proapoptotic tyrosine kinase c-Abl increases Lamp1 protein levels and autophagy flux.

Dr. Zanlungo and colleagues set out to determine whether c-Abl inhibition promotes transcription factor EB nuclear translocation, and consequently, ameliorates cholesterol accumulation in the lysosomal storage disease Niemann-Pick type C.

The investigators modulated c-Abl using a siRNA and different c-Abl inhibitors and followed transcription factor EB-green fluorescent protein subcellular localization. They also evaluated transcription factor EB tyrosine phosphorylation status by immunoprecipitation and phospho-Tyr Western blot in cells overexpressing c-Abl.

In addition, they evaluated cholesterol accumulation by filipin staining in Niemann-Pick type C1 mice and cells (Niemann-Pick type C1 null fibroblasts and Hepa 1-6 and HT22 cells treated with the U18666A drug U18) treated with c-Abl inhibitors. They used c-U18-treated hippocampal neurons to assess the participation of c-Abl.

Transcription factor EB is phosphorylated by c-Abl in tyrosine. Also, c-Abl inhibition induces transcription factor EB nuclear translocation. In addition, c-Abl inhibitors reduced cholesterol accumulation in Niemann-Pick type C1 cell models and mice.

In c-neurons treated with U18, the team observed increased Lamp1 protein levels and reduced accumulation of cholesterol.

Dr. Zanlungo concluded that the results strongly suggest that transcription factor EB tyrosine phosphorylation by c-Abl impacts transcription factor EB nuclear translocation. This phosphorylation and resulting translocation suggests a novel signaling pathway involving these two proteins. Such signaling may modulate cholesterol homeostasis in Niemann-Pick disease.

www.practiceupdate.com/c/59042

Whole Exome Sequencing Is a Good Alternative to Single-Gene and Panel Testing to Help Diagnose Lysosomal Storage Disorders

Whole exome sequencing has been found to be a good alternative to single-gene and panel testing to help diagnose lysosomal storage disorders, according to an analysis of cases of whole exome sequencing used to diagnose lysosomal storage disorders.

Alekhya Narravula, MSc, of CENTOGENE AG, Rostock, Germany, explained that whole exome sequencing is the test of choice for patients suspected of suffering from lysosomal storage disease.

Lysosomal storage diseases are a group of approximately 50 rare inherited metabolic disorders that result from defects in lysosomal function. Though each lysosomal storage disease results from a different gene mutation that translates into a deficiency in enzyme activity, they all share a common biochemical characteristic: all lysosomal disorders originate from an abnormal accumulation of substances inside the lysosome.

Lysosomal storage diseases affect mostly children, who often die at a young and unpredictable age, many within a few months or years of birth. Many other children die of this disease following years of suffering from various symptoms of their particular lysosomal storage disease.

Lysosomal storage diseases are caused by lysosomal dysfunction, usually as a consequence of deficiency of a single enzyme required for the metabolism of lipids, glycoproteins, or mucopolysaccharides.

Individually, lysosomal storage diseases occur with incidences of less than one per 100,000 individuals. As a group, however, the incidence is approximately one in 5000 to one in 10,000.

Symptoms of lysosomal storage disease vary depending on the particular disorder and other variables such as age at onset. Symptoms can range from mild to severe and can include developmental delay, movement disorders, seizures, dementia, deafness, and/or blindness. Some patients exhibit hepatomegaly and splenomegaly, pulmonary and cardiac problems, and abnormal bone growth.

Most of these disorders are autosomal-recessive, such as Niemann-Pick disease type C. A few, however, are recessive X-linked, such as Fabry disease and Hunter syndrome (mucopolysaccharidosis type II).

Though lysosomal storage diseases are well defined, they present overlapping phenotypes. Despite the availability of biochemical analyses and next generation sequencing panels, clinicians may opt for whole exome sequencing analysis, especially when unable to arrive at a specific diagnosis.

Ms. Narravula and colleagues set out to determine whether whole exome sequencing is a good diagnostic tool in lysosomal storage diseases. They retrospectively reviewed whole exome sequencing cases to date with respect to 49 lysosomal storage disorder genes. Cases were then reviewed to identify those with a confirmed or possible diagnosis.

A total of 134 cases turned out to harbor at least one reported variant (a pathogenic, likely pathogenic, or variant of unknown significance) in one of the 49 genes. A diagnosis was confirmed in 72 of 134 cases.

Sixty-six cases were homozygous/compound heterozygous for a pathogenic or likely pathogenic variant in an autosomal-recessive gene. Two cases were hemizygous for a likely pathogenic variant in an X-linked gene. Four cases were compound heterozygotes for a pathogenic variant and a variant of unknown significance.

In 45 of 134 cases, a diagnosis of lysosomal storage disease was possible, as 40 cases were homozygous/compound heterozygous for a variant of unknown significance in an autosomal-recessive gene and five cases were hemizygous for a variant of unknown significance in an X-linked gene.

In 17 cases, only one variant was detected in an autosomal-recessive gene. For six cases, deletion/duplication analysis was recommended due to significant overlap of patient symptoms with the disease.

In 11 cases, the lysosomal storage disease variant was identified in an unaffected relative, segregated in the family, and overlapped significantly with the affected index patient's symptoms.

Ms. Narravula told Elsevier's *PracticeUpdate,* "In approximately 53.7% of cases, the diagnosis of lysosomal storage disease was confirmed by whole exome sequencing. In 61.1% of these cases, lysosomal storage disorder was not in the differential diagnosis and was identified incidentally."

She continued, "The results showed that, despite a distinct phenotype and availability of the majority of biochemical tests, many patients with lysosomal storage diseases remained undiagnosed until whole exome sequencing was performed."

She added, "The high cost and lengthy time to diagnosis by stepwise single-gene testing or next generation sequencing panels; lack of local enzyme analysis; possibility of expanding the analysis to a larger set of genes; and good coverage of lysosomal storage disorder genes on exome sequencing make whole exome sequencing a good option for patients suspected of suffering from a lysosomal storage disorder." ■

www.practiceupdate.com/c/58926

No Mutations in the Three Genes Involved in BH4 Biosynthesis and Recycling were Identified in a Cohort of Patients with Dopa-Responsive Dystonia

No mutations in the three genes involved in tetrahydrobiopterin (BH4) biosynthesis and recycling were identified in a cohort of patients with dopa-responsive dystonia.

Jim Black, MD, of the University of Birmingham, UK, explained that dopa-responsive dystonia is a childhood-onset dystonic disorder (onset usually age 5–8 years) characterized by a dramatic response to low doses of levodopa.

Dopa-responsive dystonia is very rare, affecting one in two million individuals. It is more common in females than in males. Several hundred cases are in the US, 25 known cases in the UK, and fewer in Australia and New Zealand.

Characteristic symptoms are increased muscle tone and Parkinsonian features, typically absent in the morning or after rest but worsening during the day and with exertion. Owing to the rarity of the disease, children with dopa-responsive dystonia are often misdiagnosed as suffering with cerebral palsy. This misdiagnosis results in patients often living their entire childhood with the condition untreated.

When left untreated, patients often need Achilles tendon surgery by age 21 years. They will also struggle with walking, which degrades throughout the day. Power napping can provide temporary relief in untreated patients.

Improvement with sleep, with relative freedom from symptoms in the morning, and increasingly severe symptoms as the day progresses has led to the disorder being referred to as progressive hereditary dystonia with diurnal fluctuations. Yet not all patients experience such diurnal fluctuations, causing many researchers to prefer other terms for the disease.

Dopa-responsive dystonia also impairs development into adulthood, reduces balance, and reduces calf muscle development. Socially, it can result in depression, lack of social skills, and inability to find employment.

The diagnosis of dopa-responsive dystonia can be made from a typical history, a trial of dopamine medications, and genetic testing. Not all patients show mutations in the GCH1 gene (GTP cyclohydrolase I), which renders genetic testing imperfect.

Lumbar puncture is sometimes performed to measure concentrations of biopterin and neopterin, which can help determine the exact form of dopamine-responsive movement disorder. Forms are early onset parkinsonism (reduced biopterin and normal neopterin), GTP cyclohydrolase I deficiency (both decreased), and tyrosine hydroxylase deficiency (both normal).

In approximately half of cases, a phenylalanine loading test can be used to show decreased conversion from the amino acid phenylalanine to tyrosine. This process uses BH4 as a cofactor.

Decreased twitching may be noticed during REM sleep during a sleep study.

Brain MRI scanning can be used to look for conditions that can mimic dopa-responsive dystonia. For example, metal deposition in the basal ganglia can indicate Wilson's disease or pantothenate kinase-associated neurodegeneration. Nuclear imaging of the brain using position emission tomography (PET scanning) shows normal radiolabeled dopamine uptake in dopa-responsive dystonia, contrary to the decreased uptake of Parkinson's disease.

Other differential diagnoses include:

"A total of 3 of the 17 patients negative for GCH1 harbored mutations in the TH gene, two in the SPR gene, and one in the PARK2 gene.

- Metabolic disorders, such as GM2 gangliosidosis, phenylketonuria, hypothyroidism, Leigh disease
- Primarily dystonic juvenile parkinsonism
- Autosomal-recessive early-onset parkinsonism with diurnal fluctuation
- Early-onset idiopathic parkinsonism
- Focal dystonias
- Dystonia musculorum deformans
- Dyspeptic dystonia with hiatal hernia

Dopa-responsive dystonia is caused largely by autosomal-dominant mutations in the GCH1 gene (GTP cyclohydrolase1) and more rarely by autosomal-recessive mutations in the TH (tyrosine hydroxylase) or SPR (sepiapterin reductase) genes.

In addition, mutations in the PARK2 gene (parkin), which causes autosomal-recessive juvenile parkinsonism may present as dopa-responsive dystonia.

Dr. Black and colleagues set out to evaluate the relative frequency of the mutations in these genes, but also in the genes and in genes involved in the biosynthesis and recycling of BH4. They also evaluated the associated clinical spectrum.

They studied a large series of index patients (n=64) with dopa-responsive dystonia in whom dystonia improved by at least 50% after treatment with levodopa. Of these patients, 57 were classified as suffering from pure dopa-responsive dystonia and seven from dopa-responsive dystonia-plus syndromes.

All patients were screened for point mutations and large rearrangements in the GCH1 gene, followed by sequencing of the TH and SPR genes, then PTS (pyruvoyl tetrahydropterin synthase), PCBD (pterin-4a-carbinolamine dehydratase), QDPR (dihydropteridin reductase), and PARK2 (parkin) genes.

A total of 34 different heterozygous point mutations were identified in 40 patients, 6 different large deletions in 7 patients in the GCH1 gene.

Except for one patient with mental retardation and a large deletion of 2.3 Mb encompassing 10 genes, all patients exhibited stereotypical clinical features, characterized by pure dopa-responsive dystonia with onset in the lower limbs and an excellent response to low doses of levodopa. Dystonia started in the first decade of life in 40 (85%) patients and before the age of 1 year in one (2.2%) patient.

A total of 3 of the 17 patients negative for GCH1 harbored mutations in the TH gene, two in the SPR gene, and one in the PARK2 gene. No mutations were identified in the three genes involved in biosynthesis and recycling of BH4. ■

www.practiceupdate.com/c/59044

Screening for Urine Levels of Creatinine and Glycosaminoglycans Is Simple, Rapid, and Reliable in Newborns Suspected of Suffering from Mucopolysaccharidoses

Glycosaminoglycan determination in an impregnated paper urine sample has been shown to be a rapid, simple, and reliable screening for mucopolysaccharidoses that can be performed in newborns, according to the results of a 3-year, nationwide pediatric screening program.

Cristobal Colón Mejeras, MD, of the Unit for Diagnosis and Treatment of Congenital Metabolic Diseases of the National Health System, Santiago de Compostela, Spain, explained that one of the main problems of diagnosing lysosomal storage diseases is delay due to multisystem presentation. Such presentation causes pediatricians to treat isolated signs and symptoms.

Mucopolysaccharidoses (glycosominoglycans) are a group of metabolic disorders caused by the absence or malfunctioning of lysosomal enzymes needed to break down glycosaminoglycans. These long chains of sugar carbohydrates occur within cells that help build bone, cartilage, tendons, corneas, skin, and connective tissue. Mucopolysaccharides are also found in synovial fluid.

Patients with a mucopolysaccharidosis either do not produce enough of one of the 11 enzymes required to break down these sugar chains into simpler molecules, or they produce enzymes that do not work properly.

Over time, glycosaminoglycans collect in the cells, blood, and connective tissues. The result is permanent, progressive cellular damage which affects appearance, physical abilities, organ and system functioning, and, in most cases, mental development.

Mucopolysaccharidoses are part of the lysosomal storage disease family, a group of more than 40 genetic disorders that result when the lysosome organelle in animal cells malfunctions.

The lysosome may be viewed as the cell's recycling center because it processes unwanted products into other substances the cell can utilize. Lysosomes break down this unwanted matter via enzymes, highly specialized proteins essential for survival.

Lysosomal storage diseases such as mucopolysaccharidosis are triggered when the quantity of

Long-Term Migalastat Treatment Associated with Sustained Reduction in LVMi and Regression of Left Ventricular Hypertrophy in Patients with Fabry Disease

In patients with Fabry disease and amenable mutations, long-term migalastat treatment was associated with sustained reduction in left ventricular mass index and regression of left ventricular hypertrophy, results of the phase 3 FACETS and Acute venous Thrombosis: Thrombus Removal with Adjunctive Catheter-directed Thrombolysis (ATTRACT) trials report.

Ana Jovanovic, MD, of the Salford Royal Hospital and National Health Service Foundation Trust, Manchester, UK, explained that cardiac complications such as heart failure and myocardial infarction are the main cause of death in patients with Fabry disease.

Fabry disease is a rare X-linked disorder of lysosomal α-galactosidase A deficiency that causes lysosomal deposition of globotriaosylceramide. The disease causes lipid accumulation in the central nervous system, heart, kidneys, and skin. This accumulation can lead to pain, kidney failure, heart disease, and stroke. Symptoms begin at an early age. All Fabry disease is progressive and may lead to organ damage regardless of age at symptom onset.

Migalastat stabilizes lysosomal α-galactosidase A, so it can clear the accumulated disease substrate in patients with amenable mutations (an estimated 35% to 50% of patients with Fabry disease globally). An estimated 3000 individuals in the US have been diagnosed with Fabry disease, more than any other country.

Migalastat has not been approved by the FDA but was designated for fast track review in September of 2017. It has been approved in the EU, Switzerland, Israel, Australia, and Canada.

A proprietary in vitro assay (Galafold Amenability Assay) has been used to classify more than 1000 known GLA gene mutations as amenable or not amenable to treatment with migalastat. The EU label includes 331 GLA mutations that have been identified and determined to be amenable based on the assay. These

> "This analytical method was shown to be useful for early diagnosis of mucopolysaccharidosis. It was used to diagnose three cases at younger than 1 year of age. It may be extended to the entire neonatal population, opening the door to its inclusion in general newborn screening.

a particular enzyme is insufficient or the enzyme is missing altogether.

Mucopolysaccharidoses share many clinical features but severity varies. Features may not be apparent at birth but progress as storage of glycosaminoglycans affects bone, skeletal structure, connective tissues, and organs.

Neurological complications may include neuronal damage as well as pain and impaired motor function. This results from compression of nerves or spinal or peripheral nerve roots.

Dr. Colón described results of 3 years of experience using symptom-based early detection of mucopolysaccharidoses. The nationwide program was performed in an at-risk pediatric population (0–18 years of age) and was based on clinical criteria.

With the help of scientific meetings and pharmaceutical industry, Dr. Colon distributed kits with the necessary material: informed consent, clinical guide with the symptoms and warning signs to be considered, and the analytical paper by Whatman® 903 to collect biological samples of urine and blood.

From 2014 to 2017, 692 kits were requested from all regions of Spain. They saw 366 patients from 49 of 50 Spanish provinces (18% from primary care and 82% from hospitals). Urine levels of creatinine and glycosaminoglycans were determined as the main screening method in all.

Glycosaminoglycan levels exceeded the cutoff for age in 15% of samples. High glycosaminoglycan levels were confirmed in 24 patients after testing a second sample. Of the 24 cases, 17 cases of mucopolysaccharidosis were identified:

- Three mucopolysaccharidosis I
- Two mucopolysaccharidosis II
- Four mucopolysaccharidosis IIIA
- Two mucopolysaccharidosis IIIB
- Four mucopolysaccharidosis IVA
- Two mucopolysaccharidosis VI

All showed enzymatic activities below the reference value and 76% of cases were younger than 5 years of age. Glycosaminoglycan determination in an impregnated paper urine sample was shown to be a rapid, simple, and reliable screening method for mucopolysaccharidoses that can be performed in newborns.

Dr. Colón told Elsevier's *PracticeUpdate*, "This analytical method was shown to be useful for early diagnosis of mucopolysaccharidosis. It was used to diagnose three cases at younger than 1 year of age. It may be extended to the entire neonatal population, opening the door to its inclusion in general newborn screening."

He noted, "Treatment is available for mucopolysaccharidoses I, II, IVA, and VI. Clinical trials are under way of therapies for mucopolysaccharidosis III. The classic World Health Organization screening criteria proposed by Wilson and Jungner in 1968 are suitable in this context." ■

www.practiceupdate.com/c/58922

mutations represent between 35% and 50% of those diagnosed with Fabry disease.

A progressive increase in left ventricular mass index is observed across disease phenotypes, and enzyme replacement therapy has exerted variable effects on left ventricular mass index in patients with Fabry disease.

Dr. Jovanovic and colleagues set out to assess changes in cardiac parameters with long-term migalastat treatment in patients with Fabry disease in two phase 3 clinical trials.

In FACETS, 67 enzyme replacement therapy-naive patients were randomized to 6 months of migalastat 150 mg once daily or placebo, followed by 18 months of migalastat. A total of 54 patients continued migalastat in a separate open-label extension.

In ATTRACT, 60 enzyme replacement therapy-experienced patients were randomized to 18 months of migalastat or enzyme replacement therapy, followed by 12 months of migalastat. The effect of migalastat on cardiac mass was assessed by blinded echocardiography and was reported for patients in the intention-to-treat population who harbored amenable mutations.

After 18 or 24 months of migalastat treatment in FACETS (18 months for patients initially randomized to placebo, 24 months for patients randomized to migalastat), a statistically significant mean change from baseline in left ventricular mass index (7.7 g/m^2, 95% confidence interval 15.4 to 0.01; n=27) was observed.

Among patients who entered the open-label extension, further reductions were seen (month 30/36: 17.0 g/m^2, 95% confidence interval 26.2 to 7.9; n=15), including statistically significant changes in patients with left ventricular hypertrophy at baseline (20.8 g/m^2; 95% confidence interval 37.4 to 4.1; n=11); 82% (9/11) exhibited reduction and 45% (5/11), normalization of left ventricular mass index.

Similarly, left ventricular mass index was reduced in patients treated with migalastat in the ATTRACT trial. At month 18, mean changes from baseline were 6.6 g/m^2 (95% confidence interval 11.0 to 2.1; n=31) with migalastat and 2.0 g/m^2 (95% confidence interval 11.0 to 7.0; n=13) with enzyme replacement therapy.

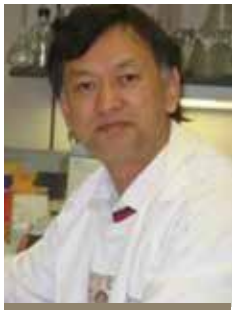
Patients treated with migalastat continued to show reductions in left ventricular mass index at month 30 (3.8 g/m^2; 95% confidence interval 8.9 to 1.3; n=30). Among patients with baseline left ventricular hypertrophy (n=13, left ventricular mass index was reduced by 9.0 g/m^2; 85% (11/13) exhibited reduction and 31% (4 of 13), normalization of left ventricular mass index.

Dr. Jovanovic concluded that, in patients with Fabry disease and amenable mutations, long-term migalastat treatment was associated with sustained reduction in left ventricular mass index and regression of left ventricular hypertrophy. ■

www.practiceupdate.com/c/59034

A Novel Surgical Reconstruction Rescues Life-Threatening Severe Tracheal Obstruction in Mucopolysaccharidoses

A novel surgical reconstruction has been shown to rescue life-threatening severe tracheal obstruction in patients with mucopolysaccharidoses, according to the findings of a case series of patients with mucopolysaccharidosis type IVA who underwent the novel reconstruction.



Dr. Shunji Tomatsu

Shunji Tomatsu, MD, PhD, of the Nemours/Alfred I. duPont Hospital for Children, Wilmington, Delaware, said patients with severe tracheal obstruction in mucopolysaccharidoses, especially mucopolysaccharidosis type IVA, are at risk of dying of sleep apnea and related complications.

Two-thirds of patients with mucopolysaccharidosis type IVA die of respiratory problems. Tracheal obstruction also leads to life-threatening complications during anesthesia as a result of the difficulty of managing the upper airway due to factors inherent in mucopolysaccharidosis.

This difficulty is compounded by that of intubation and extubation of the trachea. Though tracheostomy can address severe upper airway obstruction, lower airway obstruction, commonly associated with a narrow thoracic inlet and vascular compression, requires an alternative approach.

Dr. Tomatsu and colleagues set out to provide guidelines for earlier recognition and intervention of tracheal obstruction in these patients.

He presented a series of cases with significant tracheal obstruction that was unrecognized due to the difficulty in interpreting tracheal narrowing airway symptoms. Sagittal MRI images of the cervical spine of 28 Morquio A patients (12 ± 8.14 years of age) showed that the tracheas of 19 (67.9%) of 28 patients were at least 25% narrowed.

Narrowing worsened with age (the tracheas of all eight patients over 15 years of age were more than 50% narrowed). Eight (75%) of 28 patients were categorized as suffering from severe tracheal narrowing when images were evaluated in the neutral head and neck position.

The etiology of tracheal impingement of the brachiocephalic artery in Morquio A appeared to be due to a combination of the narrow thoracic inlet crowding structures and disproportionate growth

of the trachea and brachiocephalic artery in relation to the chest cavity. The combination leads to tracheal tortuosity.

Dr. Tomatsu saw six cases of mucopolysaccharidosis type IVA (four received enzyme replacement therapy for up to 5 years) whose near-fatal tracheal obstruction was relieved by timely surgical tracheal vascular reconstruction with dramatic resolution of respiratory symptoms.

Activities of daily living improved markedly. Tracheal narrowing, often due to impression from the crossing tortuous brachiocephalic artery, increases with age in patients with mucopolysaccharidosis type IVA.

Dr. Tomatsu concluded that greater attention to the trachea is needed when evaluating MRIs of the cervical spine as well as other imaging and clinical investigations. The goal is to establish a timely

treatment protocol to reduce mortality in this patient population.

The novel surgical procedure described could apply to other types of mucopolysaccharidosis that carry risk of severe tracheal obstruction.

Dr. Tomatsu presented results of a related study, in which he and colleagues performed the first worldwide epidemiological study of mucopolysaccharidosis.

They set out to obtain data about the epidemiology of different types of mucopolysaccharidosis in Japan and Switzerland. They compared this data with similar data from 21 other countries.

Between 1982 and 2009, 467 cases of mucopolysaccharidosis were identified in Japan. The combined birth prevalence was 1.53 per 100,000 live births. The highest birth prevalence was 0.84 for mucopolysaccharidosis type II, which accounted for 55% of all mucopolysaccharidoses.

Mucopolysaccharidosis types I, III, and IV accounted for 15%, 16%, and 10% of cases, respectively. Mucopolysaccharidosis types VI and VII were rarer and accounted for 1.7% and 1.3% of cases, respectively.

In a retrospective epidemiological data collection in Switzerland between 1975 and 2008 (34 years), 41 living patients with mucopolysaccharidosis were identified. The combined birth prevalence was 1.56 per 100,000 live births.

The highest birth prevalence was 0.46 for mucopolysaccharidosis type II, accounting for 29% of all mucopolysaccharidoses.

> "As seen with other rare genetic diseases, the frequency of mucopolysaccharidosis varies by ethnic background and/or founder effects that affect the birth prevalence of each type of the disorder.

Mucopolysaccharidosis types I, III, and IV accounted for 12%, 24%, and 24% of cases, respectively.

As seen in the Japanese population, mucopolysaccharidosis types VI and VII were rarer and accounted for 7.3% and 2.4% of cases, respectively.

The high birth prevalence of mucopolysaccharidosis type II in Japan was comparable to that seen in other East Asian countries where this type of mucopolysaccharidosis accounted for approximately 50% of all forms of mucopolysaccharidosis.

Birth prevalence was also similar in some European countries (Germany, Northern Ireland, Portugal, and The Netherlands), though the prevalence of other forms of mucopolysaccharidosis is also reported to be higher in these countries. Birth prevalence of mucopolysaccharidosis type II in Switzerland and other European countries was comparatively lower.

The birth prevalence of mucopolysaccharidosis types III and IV in Switzerland was higher than in Japan but comparable to that in most other European countries. Moreover, the birth prevalence of mucopolysaccharidosis types VI and VII was very low in both Switzerland and Japan.

Dr. Tomatsu concluded that, as seen with other rare genetic diseases, the frequency of mucopolysaccharidosis varies by ethnic background and/or founder effects that affect the birth prevalence of each type of the disorder.

Methods to identify patients with mucopolysaccharidosis are not uniform across countries. Consequently, if patients are not identified, recorded prevalence rates will be aberrantly low. ■

www.practiceupdate.com/c/58927

Differences in Carnitine Transport Across the Blood-Brain Barrier May Explain the Extremely High Male/Female Ratio in Nonsyndromic Autism

Dr. Arthur L. Beaudet

Differences in carnitine transport across the blood-brain barrier may contribute to metabolic sexual dimorphism of the brain in mammals, possibly explaining the extremely high male/female ratio in nonsyndromic autism via susceptibility to brain carnitine deficiency. This conclusion, based on results of a murine study, suggest that a Recommended Dietary Allowance for carnitine in infants should be established.

Arthur L. Beaudet, MD, of the Baylor College of Medicine, Houston, Texas, explained that he and colleagues set out to partially test the hypothesis that brain carnitine deficiency may cause 10% to 20% of all autism.

The hypothesis involves nonsyndromic or "essential" autism, with an extremely high male/female ratio in infants who are genetically normal except for common or low-penetrance genetic variants.

These infants are hypothesized to undergo a normal physical examination and structurally normal brain imaging. Dr. Beaudet characterizes this disorder as a non-Mendelian, nondysmorphic form of autism.

Unlike children with syndromic autism who are often dysmorphic, non-Mendelian, nondysmorphic autism excludes these children, as well as those with microcephaly or short stature at any age, macrocephaly at or before 3 months of age, severe hyper- or hypotonia, ataxia, abnormal reflexes, abnormal gait, prematurity, congenital malformations, dysmorphic features, or onset before 6 months of age.

It is likely but not certain that non-Mendelian, nondysmorphic autism is associated with regression, especially if acquisition of a social smile at 6–8 months of age and subsequent loss is defined as regression. Regression was reported in a male with TMLHE deficiency and very low plasma carnitine (Ziats et al, 2015).

The infants are hypothesized to develop deficiency of carnitine and perhaps other nutrients in the brain. The deficiency causes autism that may be amenable to early reversal and prevention through dietary carnitine supplementation.

Dr. Beaudet proposed a mixed, common gene variant – environment hypothesis with diet, minor illnesses, microbiome, and drugs as possible risk modifiers. Dr. Beaudet's team searched for a carnitine-related explanation for the high male/female ratio of autism.

They found that a gene on the X chromosome in humans and mice (SLC6A14/ Slc6a14) likely escapes random X-inactivation due to the absence of differential methylation on the inactive X chromosome in both mice and humans.

The SLC6A14 protein is an amino acid and carnitine transporter that functions at the blood-brain barrier. Lack of X-inactivation could lead to greater expression in females than males at the blood-brain barrier and limit transport of carnitine across the blood-brain barrier in boys compared to girls.

The investigators assessed transport across the blood-brain barrier in mice by tail vein injection of radioactive carnitine. After 4 h, transport to the brain was greater in wild-type female mice than in male mice, likely due to the lack of X-inactivation of Slc6a14.

Transport of radioactive carnitine across the blood-brain barrier is reduced in female and male Slc6a14 null mutants compared to wild-type mice.

Dr. Beaudet concluded that Slc6a14-mediated transport across the blood-brain barrier may be greater in female than in male mice.

He proposed that differences in carnitine transport across the blood-brain barrier may contribute to metabolic sexual dimorphism of the brain in mammals, possibly explaining the extremely high male/female ratio in nonsyndromic autism via susceptibility to brain carnitine deficiency.

Perhaps the lack of a Recommended Dietary Allowance for carnitine in infants should be reviewed.

Brain deficiency of carnitine, and perhaps other micronutrients including polyunsaturated fatty acids, may cause non-Mendelian, nondysmorphic autism. Early treatment with carnitine and other micronutrient supplementation may benefit recently symptomatic children and prevent recurrence risk in both families and in the general population.

A prevention trial should be carried out in families with infant siblings. A method should be sought to measure brain carnitine noninvasively in vivo in children using imaging methods.

Dr. Beaudet told Elsevier *Practice Update,* "I am very excited to have published the full description of our hypothesis, If the hypothesis is correct, there may be a global opportunity to reduce the frequency of autism by modifying infant nutrition."

He added, "I plan to experiment and gather data in mice and humans in an attempt to determine whether the hypothesis is valid." ■

www.practiceupdate.com/c/58921

Ingestion of Triheptanoin-Containing Chow Improves Exercise-Associated Cardiac Muscle Anaplerosis in Murine VLCAD Deficiency

A role has been suggested for administration of anaplerotic substrates in murine models of fatty acid oxidation disorder according to the findings of a prospective comparative study.

Fatty acids represent an important source of energy in periods of catabolic stress related to increased muscular activity or fasting or febrile illness, in which as much as 80% of energy to the heart, skeletal muscles, and liver may be derived from them.

They play an important role in the neonate due to limited glycogen reserves and high metabolic rate. Fatty acid oxidation produces acetyl coenzyme A, which supplies energy to other tissues when glycogen stores are depleted.

> "There may be a role for administration of anaplerotic substrates in murine models of fatty acid oxidation.

Medium and short fatty acids are transported directly into the cytosol and mitochondria. Long-chain fatty acids are conjugated to carnitine and transported across the mitochondrial membrane and released as acetyl coenzyme to be used in the β-oxidation path.

Mitochondrial fatty acid β-oxidation disorders are a heterogeneous group of approximately 20 defects in fatty acid transport and mitochondrial β-oxidation. They are inherited as autosomal-recessive disorders and present in a wide range of clinical phenotypes.

Presentation can be either neonatal with hyperammonemia, transient hypoglycemia, metabolic acidosis, cardiomyopathy, and sudden death; or late in onset with neuropathy, myopathy, and retinopathy. Most cases are identified using newborn screening by mass spectrometry of blood spots.

Pregnancies of mothers heterozygous for fatty acid β-oxidation disorders have been associated with development of severe preeclampsia, acute fatty liver of pregnancy, and hemolysis, elevated liver enzymes, low platelets (HELLP) syndrome in mothers and intrauterine growth retardation in infants.

Mitochondrial fatty acid oxidation disorders are composed of four groups:

1. Disorders of the entry of long-chain fatty acids into mitochondria
2. Intramitochondrial β-oxidation defects of long-chain fatty acids affecting membrane bound enzymes
3. β-oxidation defects of short- and medium-chain fatty acids affecting enzymes of the mitochondrial matrix
4. Disorders of impaired electron transfer to the respiratory chain from mitochondrial β-oxidation

Garen Gaston, MS, of Oregon Health Science University, Portland, explained that dietary odd-chain fatty acid supplementation has been suggested as a method to increase citric acid cycle intermediate pools and energy metabolism in subjects with long-chain fatty acid oxidation disorders such as very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency.

Mr. Gaston and colleagues set out to investigate citric acid cycle intermediate depletion after exhaustive exercise and the ability of triheptanoin to increase citric acid cycle intermediates in murine VLCAD.

Wild-type or VLCAD knockout mice fed normal chow were monitored by indirect calorimetry at rest and during exercise. VLCAD knockout mice were exercised at 60% VO2 max to exhaustion or up to 60 minutes on a treadmill. Wild-type animals were similarly exercised, and citric acid cycle intermediates was measured in cardiac tissue by stable isotope dilution mass spectrometry for targeted metabolomics.

To investigate the effects of odd-chain supplementation at rest and during exercise stress, wild-type or VLCAD knockout mice were fed chow supplemented with triheptanoin or medium-chain triglycerides at 30% of energy for 4 weeks. Indirect calorimetry and cardiac citric acid cycle intermediates were measured as described above.

Resting VLCAD knockout mice fed normal chow exhibited a similar respiratory exchange ratio but lower VO2, suggesting similar substrate utilization but lower energy expenditure than wild-type mice.

VLCAD knockout mice fed triheptanoin or medium-chain triglycerides exhibited lower respiratory exchange ratio (increased fat oxidation) and higher VO2 than their counterparts fed normal chow.

This suggested that both oils are oxidized and increase energy expenditure. Exercised VLCAD knockout mice fed normal chow became exhausted far sooner than wild-type mice, but VLCAD knockout mice fed triheptanoin or medium-chain triglycerides became exhausted at a similar rate to wild-type animals.

Exercised VLCAD knockout mice exhibited a lower succinate concentration in cardiac muscle on exhaustion than exercised wild-type and rested VLCAD knockout animals. This suggested decreased anaplerosis with prolonged exercise.

Exercised VLCAD knockout mice fed triheptanoin, however, exhibited higher cardiac malate and succinate than exercised VLCAD knockout mice fed medium-chain triglycerides. This suggested that anaplerosis had been partially restored in triheptanoin-supplemented animals.

Interestingly, metabolomic studies demonstrated accumulation of long odd-chain fats in triheptanoin-fed animals. This suggested that at least a portion of ingested triheptanoin had been elongated to longer-chain fatty acids rather than being oxidized exclusively.

Mr. Gaston told Elsevier *PracticeUpdate*, "VLCAD knockout mice exhibited decreased cardiac succinate following exhaustive exercise. Triheptanoin supplementation led to increased cardiac malate and succinate."

"There may be a role for administration of anaplerotic substrates in murine models of fatty acid oxidation," he added. ■

www.practiceupdate.com/c/58924

Founder Mutation and New Diagnostic Biomarker of PLA2G6-Associated Neurodegeneration Are Identified

Two mutations associated with neurodegenerative disease: the founder mutation and a new diagnostic biomarker of PLA2G6-associated neurodegeneration (PLAN) have been identified in a large North African cohort, according to a new report.

Dr. Ichraf Kraoua

Ichraf Kraoua, MD, of the National Institute Mongi Ben Hamida of Neurology of Tunis, Tunisia, explained that mutations in the PLA2G6 gene cause PLAN, a spectrum of neurodegenerative conditions including infantile, childhood, and adult-onset forms.

PLA2G6 encodes the group VI calcium-independent phospholipase A2, which hydrolyzes glycerophospholipids to lysophospholipids and free fatty acids. This enzyme is essential for membrane homeostasis and repair and for maintenance of mitochondrial membranes.

"PLAN is often misdiagnosed," Dr. Kraoua told *PracticeUpdate*. "We collected a large cohort in Tunisia but we were not able to confirm it molecularly. We collaborated with Enza Maria Valente, MD, PhD, of Casa Sollievo della Sofferenza-Mendel Institute, Rome. She gave us the opportunity to confirm our patients and then to discover the founder mutation, initially in 17 patients."

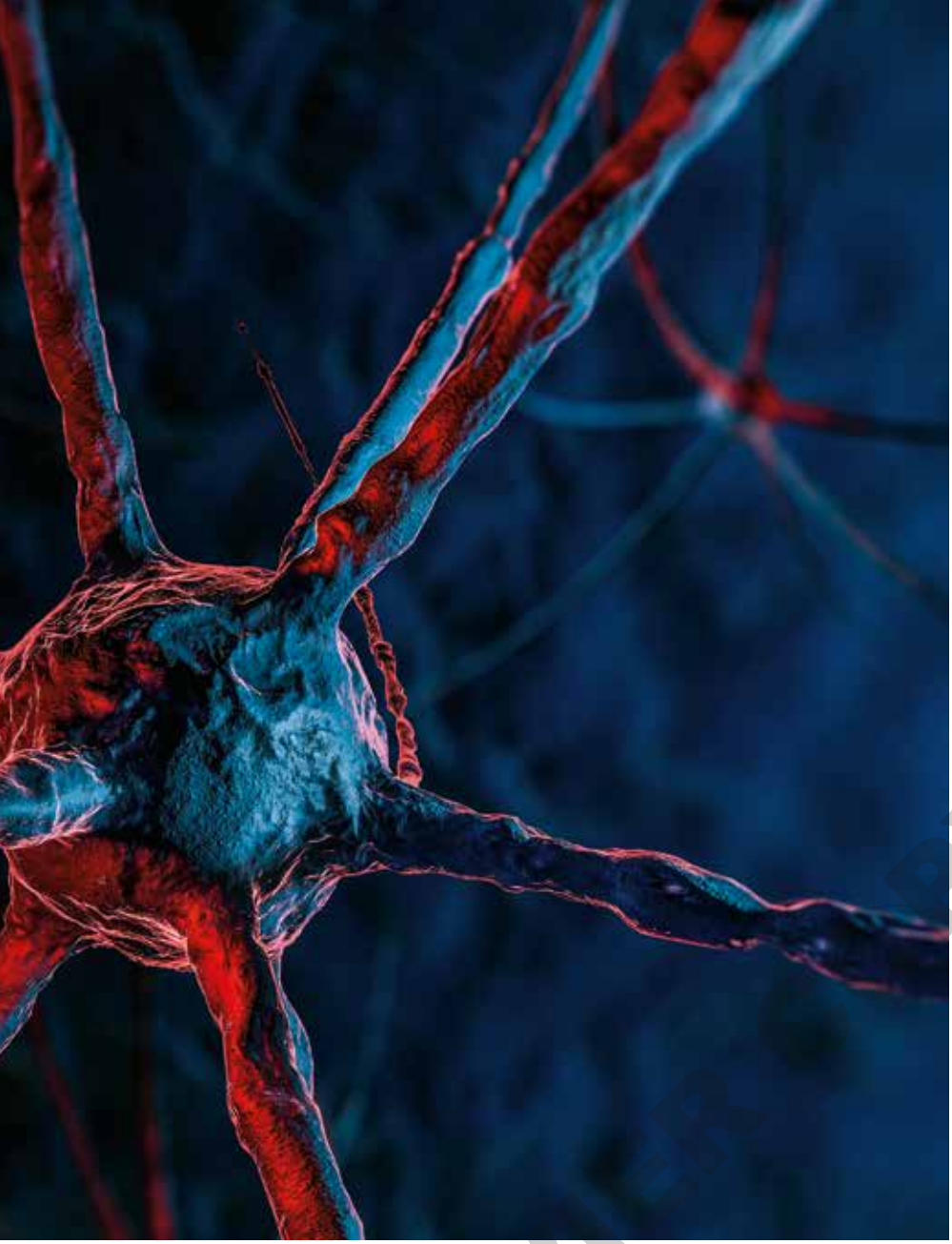
"After that, we considered this mutation the first in Tunisian patients diagnosed with PLAN, and we confirmed the remaining patients in Tunisia," she added.

Thirty North African (26 Tunisian, three Algerian, and one Libyan) patients suspected clinically of suffering from infantile-onset PLAN underwent clinical, biological, neurophysiological, and neuroimaging examinations and PLA2G6 sequencing.

Twenty-nine children exhibited the commonest form of infantile-onset PLAN, with early onset of psychomotor regression, hypotonia, pyramidal and cerebellar signs, and abnormal ocular movements. The phenotype was highly homogeneous, with rapid development of severe spastic tetraparesis, cognitive impairment, and optic atrophy.

"Regarding the diagnostic biomarker," Dr. Kraoua said, "we performed systematic routine biological screening for all patients. We discovered elevation in aspartate transaminase and high lactate dehydrogenase in all patients, even at an early stage. We also compared our results with an Italian cohort and they exhibited the same abnormalities."

Of 28 patients who underwent routine biochemical testing, all exhibited mildly increased levels of aspartate aminotransferase and lactate dehydrogenase, even at early stages of the disease.



Neuroimaging showed cerebellar atrophy and claval hypertrophy to be the commonest and earliest signs. Cerebellar cortex hyperintensity and pallidal iron deposition were later findings.

Motor or sensory motor axonal neuropathy was frequent (20 of 29 patients). Fifteen patients from 10 families shared the same mutation (p.V691del).

Neuroimaging showed cerebellar atrophy and claval hypertrophy to be the commonest and earliest signs. Cerebellar cortex hyperintensity and pallidal iron deposition were later findings. Motor or sensory motor axonal neuropathy was frequent (20 of 29 patients).

Fifteen patients from 10 families shared the same mutation (p.V691del). One patient fitted the diagnosis of the much rarer childhood-onset PLAN.

The following finding seems specific to PLAN: 21 patients with neurodegeneration with brain iron accumulation mutated in other genes (16 PANK2, two C19orf12, two COASY, one WDR45) and 56 patients with distinct neurological conditions such as mental retardation, dystonia, cerebral palsy, epilepsy, mitochondrial encephalopathy. All exhibited normal aspartate transaminase values, while lactate dehydrogenase was mildly elevated in nine only.

Different from alanine transaminase and creatine phosphokinase, aspartate transaminase is widely expressed also in neuronal cells. It exists in two main isoforms, cytoplasmic and mitochondrial.

Intriguingly, besides its enzymatic activity, mitochondrial aspartate transaminase was found to be a plasma membrane binding protein essential for free fatty acid uptake. This finding led to speculation that, in PLA2G6-mutated patients, widespread mitochondrial damage and/or the dysfunctional metabolism of free fatty acids may result in abnormal release of mitochondrial aspartate transaminase from damaged cells.

> "Elevated aspartate transaminase/alanine transaminase ratio associated with high lactate dehydrogenase values may be considered a potential supportive biomarker to point toward a diagnosis of PLAN, even in very early stages of the disease.

Lactate dehydrogenase is a widespread cytoplasmic enzyme that catalyzes the anaerobic conversion of pyruvate to lactate, whose levels usually increase in the presence of tissue and cellular damage and in many cancers. Thus, its rise in PLAN may be less related to diffuse neuronal damage specifically.

The diagnostic workup of patients suspected of suffering from PLAN includes brain imaging, neurophysiological, and ophthalmological assessment. All these tests can be inconclusive at onset. In this light, the observation of an elevated aspartate transaminase/alanine transaminase ratio and lactate dehydrogenase may represent a potential supportive biomarker of PLAN, prompting PLA2G6 genetic testing even in early stages of disease or in atypical forms.

One patient fitted the diagnosis of the much rarer childhood-onset PLAN. Despite early onset (18 months of age), clinical progression of this patient was slower, with behavioral disturbances and dystonia. The patient carried a missense variant predicted to be less deleterious.

Dr. Kraoua concluded that elevated aspartate transaminase/alanine transaminase ratio associated with high lactate dehydrogenase values may be considered a potential supportive biomarker to point toward a diagnosis of PLAN, even in very early stages of the disease. The p.V691del mutation is founder and should be considered a priority in North African patients.

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Experience With Hematopoietic Stem Cell Transplantation for Mucopolysaccharidosis Type IH Has Not Been Favorable in Brazil

Dr. Carolina Fischinger Moura de Souza

The outcome of hematopoietic stem cell transplantation for mucopolysaccharidosis type IH has not been favorable in the experience of three Brazilian centers.

Carolina Fischinger Moura de Souza, MD, PhD, of the Hospital de Clínicas de Porto Alegre, Brazil, explained that hematopoietic stem cell transplantation has been successful in Hurler syndrome (mucopolysaccharidosis type I severe form, or mucopolysaccharidosis type IH).

Clinical features of Hurler syndrome include coarse facies, corneal clouding, mental retardation, hernias, dysostosis multiplex, and hepatosplenomegaly. Children with Hurler syndrome appear normal at birth and develop the characteristic appearance over the first years of life.

In 1987, Wraith et al reviewed 27 patients with Hurler syndrome, 10 of whom were evaluated prior to biochemical diagnosis. Diagnosis was established at a mean age of 21 (range 5–63) months. Seventeen of the children (63%) came to clinical attention with a hernia prior to the diagnosis of Hurler syndrome. The average age at death was 6.25 (range 1.3 to 10.9) years in their series of 27 patients.

In 1995, Cleary and Wraith described the presenting features of 39 patients with mucopolysaccharidosis type IH. Mean age at diagnosis was approximately 9 months. An earlier age at diagnosis was likely to lead to better results following therapy such as bone marrow transplantation. The investigators concluded that clinical features that should arouse suspicion of mucopolysaccharidsosis type IH include frequent ear, nose, and throat surgery and recurrent hernias.

In 1993, McDowell et al described a family in which siblings with comparable deficiencies of a-L-iduronidase exhibited different clinical severity and disease progression. The cases underscored the need for caution in counseling and the limitations of using siblings as controls in evaluating treatment outcomes.

> "Though the outcomes of hematopoietic stem cell transplantation were not optimal for mucopolysaccharidosis type 1H in these Brazilian centers, we are in favor of the procedure.

Hematopoietic stem cell transplantation corrects the enzyme defect in white blood cells of patients with mucopolysaccharidosis type IH, though it does not provide complete clinical recovery.

Fatal complications may be prevented, and children with mucopolysaccharidosis type IH treated with hematopoietic stem cell transplantation generally live longer than untreated children.

Dr. Fischinger Moura de Souza and colleagues reported on experience in three Brazilian centers. Hematopoietic stem cell transplantation was performed in eight patients with mucopolysaccharidosis type IH over a period of 6 years (2010–2016): four males and four females were transplanted in two centers in Southern Brazil (Curitiba and Porto Alegre).

All patients were homozygous for the p.W402X mutation. Age at diagnosis ranged from 1 to 22 months. Age at hematopoietic stem cell transplantation ranged from 8 to 28 months. Seven of eight patients received enzyme replacement therapy with laronidase from 10 to 24 months before hematopoietic stem cell transplantation.

In five of seven cases, the donor was an HLA-matched unrelated volunteer. The conditioning regimen consisted of busulfan and cyclophosphamide with mesna. Rabbit-derived antithymocyte globulin was used to prevent graft rejection in combination with the conditioning regimen only in hematopoietic stem cell transplantation from unrelated donors.

Primary graft failure was observed in six of eight patients. Three patients have died, and one received a second transplant. The primary cause of death was infection in two cases and disease progression in third, after primary graft failure.

Of the three living patients, one received three transplants and suffered from severe disease progression after graft failure. The other harbors functional grafts and a favorable long-term outcome after a median follow-up duration of 5 years.

This patient displays mixed chimerism (30%). Despite low chimerism, patients have experienced improvement in motor skills, language, and brain lesions. Dysostosis multiplex has progressed.

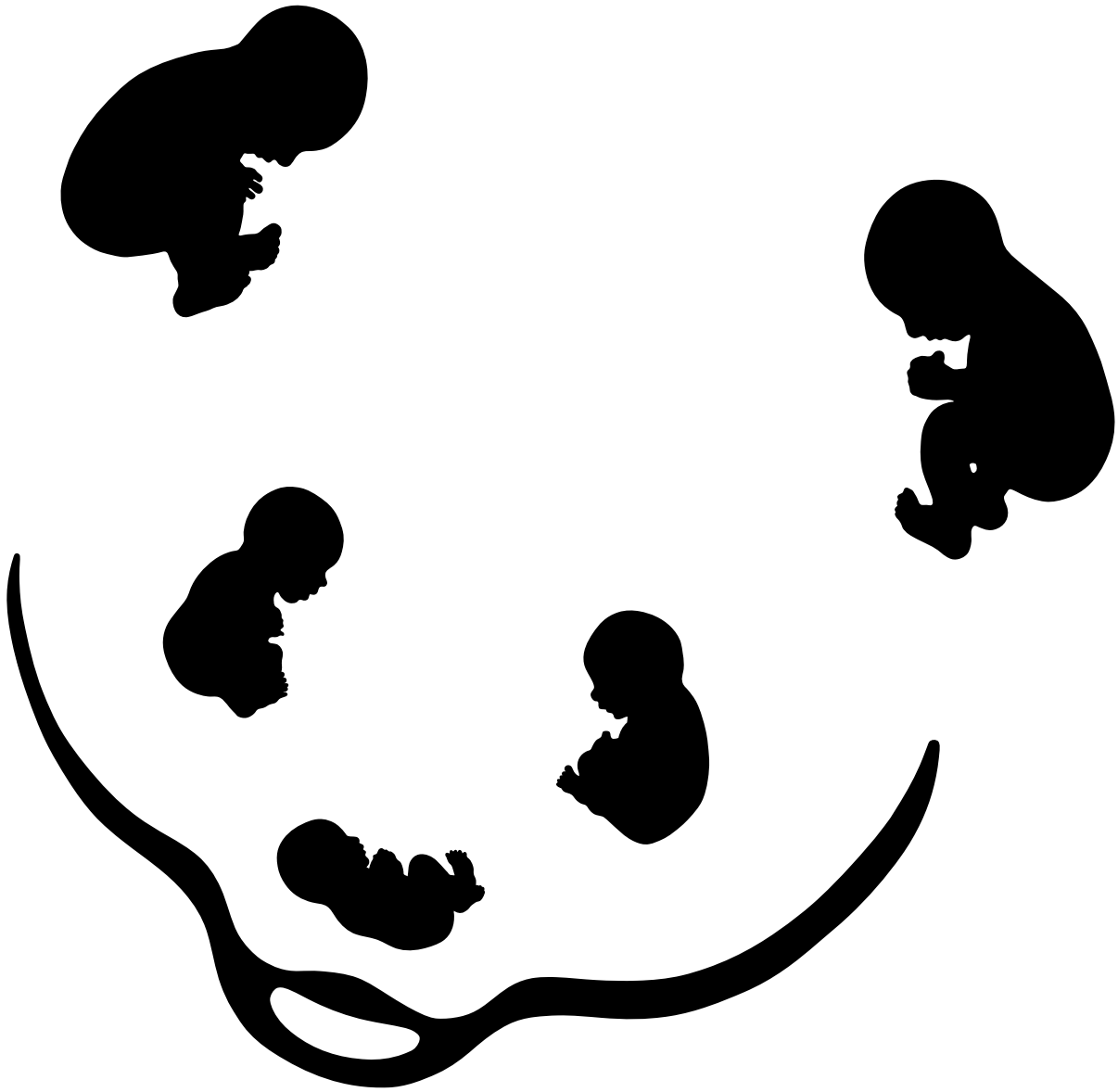
Dr. Fischinger Moura de Souza concluded that the outcome of hematopoietic stem cell transplantation for mucopolysaccharidosis type IH has not been favorable in the experience of these three Brazilian centers. Reasons for the unfavorable outcomes are probably:

- Patients with mucopolysaccharidosis were diagnosed late
- The waiting time for hematopoietic stem cell transplantation was long
- A unified protocol with indications for the procedure and guidelines for follow-up was lacking

Patients with a favorable outcome, however, have noticed stabilization of their disease progression and normalized biochemical parameters and their neurological development has been better. Bone dysplasia, however, has progressed.

Dr. Fischinger Moura de Souza told Elsevier's *PracticeUpdate*, "Though the outcomes of hematopoietic stem cell transplantation were not optimal for mucopolysaccharidosis type 1H in these Brazilian centers, we are in favor of the procedure. Its duration needs to be improved, as well as diagnosis." ■

www.practiceupdate.com/c/59033



Not too rare to care

The rarity of MPS counts against early diagnosis[1–3]

Collectively affecting only one in 22,500 births,[3] the very rarity of mucopolysaccharidoses (MPSs), together with a varied and subtle clinical presentation, make early diagnosis difficult.[1,2,4] This can expose patients to irreversible organ damage that treatment may prevent.[1,2,4]

To assist in the diagnosis of rare diseases like MPS, Sanofi Genzyme is providing paediatricians with access to a highly-sophisticated differential checklist built on a database of 6,000 diseases.

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Make **Rare Diseases** stand out

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3. Muenzer J *et al. Pediatrics* 2009; 123 (1): 19-29. 4. Burton BK, Giugliani R. *Eur J Pediatr* 2012; 171: 631-9.

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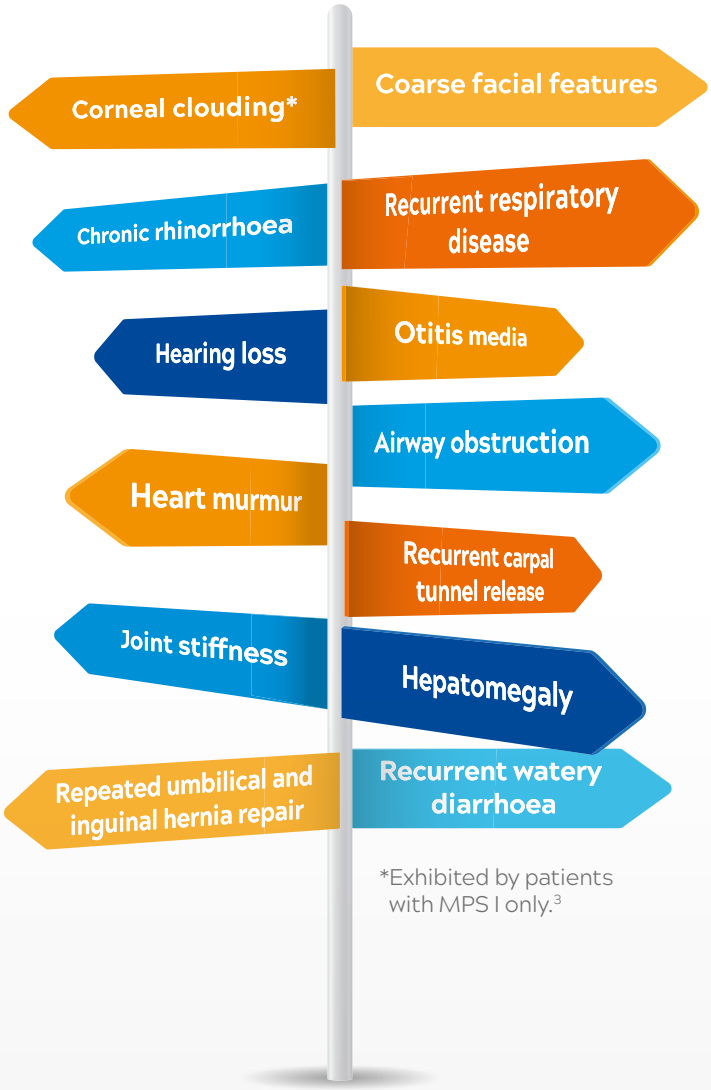
SANOFI GENZYME

THE SIGNS & SYMPTOMS OF MPS I OR II COULD BE UNFOLDING IN FRONT OF YOU... BUT WILL YOU SEE THEM IN TIME?[1-3]

Left untreated, patients can face a reduced life expectancy of as little as 10 years.[2,4]



*Exhibited by patients with MPS I only.[3]

TREATMENTS ARE AVAILABLE, SO DON'T DELAY. SUSPECT MPS TODAY.

If you suspect MPS I or II proceed to order a urinary metabolic screen (including a urinary GAG test), or refer to your local metabolic physicians for further information and advice.**

**Aim to include as much clinical information as possible when requesting a metabolic screen.

MPS: mucopolysaccharidosis; GAG: glycosaminoglycans. Images reproduced with permission.

References: 1. Muenzer J *et al*. *Orphanet Journal of Rare Diseases* 2017; 12: 82-91. **2.** Beck M *et al*. *Genet Med* 2014; 16: 759-65. **3.** Burton B & Giugliani R. *Eur J Pediatr* 2012; 171: 631-9. **4.** Wraith J *et al*. *Genet Med* 2008; 10: 508-16.

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