



Inherited disorders, metabolic disorders and rare diseases

Conclusions

Due to differences in amino acids profile between girls with Turner syndrome and obese girls, it is suggested that obesity in patients with Turner syndrome may not be associated with BCAAs and aromatic amino acids metabolism.

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W172

Amino acids profile in children with Turner syndrome

J. Bugajska^a, J. Berska^a, M. Wójcik^b, J.B. Starzyk^b, K. Sztefko^a

^aDepartment of Clinical Biochemistry, Institute of Pediatrics, Jagiellonian University, Medical College, Krakow, Poland

^bDepartment of Pediatric and Adolescent Endocrinology, Chair of Pediatrics, Institute of Pediatrics, Jagiellonian University, Medical College, Krakow, Poland

Background-aim

Girls with Turner syndrome are at increased risk of developing the insulin resistance and the coronary artery disease as a result of the higher frequency of hypertension and obesity. On the other hand it is known that obesity is associated with an increase of serum levels of branched-chain (BCAAs: valine, leucine and isoleucine) and aromatic amino acids (phenylalanine, tyrosine). The aim of the study was to compare amino acids profile in girls with Turner syndrome and girls with obesity.

Methods

The study included 46 girls with Turner syndrome (study group, mean age: 12.4 ± 4.2 years) and 22 girls with obesity (control group, mean age: 14.0 ± 2.9 years). Body mass index (BMI) was expressed as SDS (Standard Deviation Score). The mean value of BMI SDS in study group was 4.38 ± 2.22 and in control group was 1.00 ± 1.71 . Free plasma amino acids are measured by the fully validated, highly selective liquid chromatography-tandem mass spectrometry (Agilent Technologies, Jsem). The following amino acids were determined: aspartic acid, glutamic acid, serine, asparagine, glycine, glutamine, taurine, histidine, citrulline, threonine, alanine, arginine, proline, tyrosine, methionine, valine, isoleucine, leucine, phenylalanine, tryptophan, ornithine and lysine.

Results

The mean values of BCAAs, methionine, phenylalanine, lysine, tryptophan, histidine, tyrosine, aspartic acid, alanine and ornithine were significantly lower in the study group than in the control ($p < .005 - p < .000001$). Strong significant positive correlation between BCAAs, alanine, tyrosine, phenylalanine and BMI SDS in the patients with obesity ($r = 0.58, p < .0046$; $r = 0.42, p < .049$; $r = 0.59, p < .0042$; $r = 0.51, p < .0144$; $r = 0.54, p < .0098$; $r = 0.43, p < .0448$; respectively) has been found. In contrast, there was no correlation between any of measured amino acids and BMI SDS in the girls with Turner syndrome.

W173

Circulating coenzyme Q10 (COQ10) in patients with inborn error of metabolism treated by low protein diet

M. Chtourou, A. Imbard, O. Rigal, J.F. Benoist

Robert Debré Hospital, Biochemistry-Hormonology Laboratory, Paris, France

Background-aim

The CoQ10 is known by its role in bioenergetics as a cofactor of the respiratory chain and as an antioxidant. It can be produced endogenously. The first stages of this biosynthesis are common with those of the cholesterol. In addition, a small quantity is daily supplied by the food, mainly by natural proteins. Consequently, the deficit in CoQ10 can be primitive, caused by genetic disorders of biosynthesis, or secondary due to insufficient dietary intake.

We investigated the impact of a low protein diet, in patient with inborn errors of metabolism on plasma coenzyme Q10 concentrations.

The aim of our study is to compare the CoQ10's plasma concentrations in patients with an inborn error of metabolism treated with a low protein diet with controls.

Methods

We measured plasma concentrations of CoQ10 by tandem mass spectrometry and total cholesterol in three sub groups of inborn error of metabolism (organic aciduria or the urea cycle disorders $n = 16$, maple syrup urine disease (MSUD) $n = 10$ and phenylketonuria $n = 10$) treated by low protein diet versus a group of 100 controls.

Results

The plasma concentration of the CoQ10 was significantly lower in the subgroup of MUSUD than its concentration in the other subgroups of low protein diet patients or controls. We did not observe any difference in the ratio CoQ10/Cholesterol.

Conclusions

The significant decrease of the CoQ10's plasma concentrations observed in the sub group of MUSD suggests a direct role of leucine on the lipid metabolism, in particular that of the cholesterol biosynthesis.

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W174

A novel heterozygous missense variant in GNE: Diagnosis of SIALURIA

M. Fabre Estremera^a, J. Liro Armenteros^b, P. Jimenez-Arriscado^c, E. Calderón^d, J.M. Guerrero Montavez^c, H. Macher Manzano^c

^aClinical Biochemistry Department, Hospital Clínico Universitario Lozano Blesa, Zaragoza, Spain

^bClinical Biochemistry Department, Hospital Universitario Virgen de la Macarena, Sevilla, Spain

^cClinical Biochemistry Department, Hospital Universitario Virgen del Rocío, Sevilla, Spain

^dDepartment of Internal Medicine, Hospital Universitario Virgen del Rocío, Sevilla, Spain

Background-aim

Sialuria is a rare autosomal dominant, inborn error of metabolism characterized by constitutive overproduction, cytoplasmic accumulation and urinary excretion of free sialic acid. This disease is the consequence of feedback failure inhibition for the rate-limiting enzyme in the sialic acid synthesis pathway, UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE/MNK). Sialic acids play very important functions in cellular recognition. Moreover, sialic acids are important in the development of nervous system, by mediating cell-cell recognition. Reduced sialylation levels can result in neuromuscular disorders. The estimated prevalence of Sialuria is 1 < 1,000,000 in live births. Up to now, ninth cases had been published worldwide, all with missense variants of GNE. Here we report a case of Sialuria with a novel heterozygous missense variant in GNE.

Methods

An 18-years-old patient is monitored due to epilepsy. At the age of 18 months he had an episode of tonic-clonic seizures replicated over months. He attended schools for children with mild disabilities and slow in manipulations. In Secondary school he was diagnosed as TDAH. In suspicion of a rare diseases, a genetic testing of Metabolopathies was performed. DNA was extracted from blood samples by the MagNAPure system (Roche) and has been sequenced with a custom design from Ion Ampliseq ThermoFisher S5 Ion Torrent Platform.

Results

As a result, we found a heterozygous missense variant c.1055G>A p.Gly352Asp in GNE. Not previously described but with pathogenic prediction in silico analysis systems. Despite the fact that no increase of urine sialic acid has been found his clinical symptoms could be explained by this mutation, and sialuria is not always present in other cases.

Conclusions

We report a novel heterozygous missense variant in GNE with massive sequence analysis and symptoms correlate in the patient. These facts could be explained assuming a disruption in the wild-type conformation of GNE/MNK, where a glycine is substituted by aspartic acid leading to changes in the protein charge. The patient diagnosis has been delayed until his 18 years old because of the difficult identification of rare diseases where a no screening method exists, but nowadays massive sequence approach could save a lot of time to these minority diseases.

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W175

Difficulties in the diagnosis of galactokinase deficiency. Urine usefulness for the diagnosis and control

E. Fernández^a, D. Rausell^b, S. Moliner^c, I. Vitoria^b, P. Briones^d, M.J. Coll^{c,d}

^aDepartment of Biochemistry and Molecular Genetics (CDB), Hospital Clínic, Barcelona, Spain

^bDivision of Congenital Metabolopathies, University and Polytechnic Hospital La Fe, Valencia, Spain

^cInborn Errors of Metabolism (IBC), Department of Biochemistry and Molecular Genetics (CDB), Hospital Clínic, Barcelona, Spain

^dCSIC, Barcelona, Spain

Background-aim

Galactokinase (GALK) deficiency is one of the three inborn errors of galactose metabolism leading to hypergalactosemia. The main clinical manifestation is the development of cataracts during the first months of life, caused by accumulation of galactitol in lens. Early identification and treatment is essential to reverse cataracts and improve quality of life. Galactitol plasma concentration is the most informative marker, but its quantification is only performed in high specialized laboratories. Because of difficulties evaluating galactitol, galactose determination in plasma is the fastest and more efficient method for GALK diagnosis. However, due to the fact that plasma galactose comes from diet, the analysis of this metabolite has limitations and may lead false negative (FN) results.

Methods

We present a case report and the difficulties for its diagnosis. A 2-year-old male with cataracts who had presented an episode of prolonged gastroenterocolitis and hypoglycaemia. Blood samples were referred to our laboratory to determine galactose levels in plasma by spectrophotometry.

Results

Galactose levels in plasma resulted within normal limits. Due to the high clinical suspicion of galactose metabolism disorder, oligosaccharide analysis in urine by thin-layer chromatography was performed, showing increased levels of galactose excretion. Although in a second plasma sample the galactose value was still normal, the quantitative determination of galactose in urine, which was taken in parallel, was increased (23.8 μmols/μmols creatinine; age matched controls: 0.05–0.08 μmols/μmols creatinine). GALK activity analysis

showed clear deficiency in the patient's erythrocytes and intermediate activity in his parents, as befits their character as heterozygotes. After incorporation a free galactose diet, the values in urine, completely normalized.

Conclusions

In the presented case, the single determination of galactose in plasma gave completely normal result and did not allow us to detect the GALK deficiency. This FN result can be explained by an inappropriate sample collection, which possibly was taken in the early morning and fasting. It is very important to take into account the time of blood sample collection, which should be done within 3–4 h after ingestion of milk. This finding leads us to propose some changes in the protocol and confirms the importance of pre analytical phase and its impact on the quality of laboratory results.

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W176

Evaluation of the INNO-LiPA CFTRiagi test principle

G. Mersch, B. De Decker, C. Dobbels, L. Tremmerie, V. Kostanjevecki
Fujirebio-Europe NV, Technologiepark 6, Gent, Belgium

Background-aim

Nearly 2000 cystic fibrosis (CF)-associated mutations have been reported across the cystic fibrosis transmembrane regulator (CFTR) gene. Thus, efficient screening for CF requires an assay that can detect multiple mutant and wild-type sequences of the CFTR gene. Unlike cumbersome and more expensive sequencing-based approaches, reverse-hybridization (Rev-Hyb) assays, such as line probe (LiPA) assays, are easy to perform without expensive instrumentation. However, the number of probe lines that can be immobilized on a support is limited. To comply with the newest guidelines and regulations, currently 5 LiPA assays need to be performed.

Current LiPA assays need to increase the simultaneous detection of CFTR mutations while reducing the number of test runs.

Methods

The INNO-LiPA CFTRiagi (LiPA CFTRiagi) is used for the simultaneous analysis of 88 CFTR mutations (1 extra mutation) based on Rev-Hyb assay. An amplification product of the CFTR gene is hybridized with pools of probes immobilized on the LiPA CFTRiagi strip. Only when necessary, a second hybridization reaction on the relevant additional CFTR LiPA strip would follow. In this case, the same amplification product of the LiPA CFTRiagi can be reused to test the individual probes that are comprised in the positive pool(s) from the LiPA CFTRiagi strip.

To further reduce the number of LiPA CFTR assays, the LiPA CFTRiagi strip contains, in addition to the pooled mutation lines, a series of 12 individual mutation lines and corresponding wild type lines of the most frequent CFTR mutations, without requiring an additional hybridization test.

Results

The LiPA CFTR portfolio consists of 5 different typing kits each designed for a particular set of individual CFTR mutations. To test for all 87 mutations, 5 amplification and hybridization assays must be

performed. Since the goal for CFTR testing is to find 2 mutations in affected patients, CFTRiagi reduces the complete mutation analysis to a maximum of 3 hybridization assays using 1 amplification. Because the most frequent mutations are also present on this LiPA CFTRiagi strip, this number of hybridization assays will be reduced to only 1 in 90 to 95% of the cases.

Conclusions

Using LiPA CFTRiagi, the determination of 88 CFTR mutations can be reduced from 5 assays to 1 (in 90 to 95% of the cases) or maximum 3 assays.

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W177

The decision making levels of urine tetrasaccharide for diagnosis of Pompe disease

E. Canbay^a, M. Vural^a, S. Kalkan Ucar^c, E. Demirel Sezer^a, H. Karasoy^b, A. Yuceyar^b, M. Coker^c, E. Sozmen^a

^aDepartment of Medical Biochemistry, Ege University, Faculty of Medicine, Turkey

^bDepartment of Neurology, Ege University, Faculty of Medicine, Turkey

^cDepartment of Pediatric Metabolism, Ege University, Faculty of Medicine, Turkey

Background-aim

Recently, urinary excretion of the tetrasaccharide 6-(α -D-glucopyranosyl-maltotriose (Glc4) has been proposed as a marker for diagnosis and monitoring of Pompe disease. We aimed to determine the reference intervals and reliable decision making levels of urine tetrasaccharide concentrations for diagnosis of Infantile- and Late-Onset Pompe patients.

Methods

9 patients with Pompe disease (5 of them with Late-onset Pompe disease) and 226 healthy controls were included into this study (between ages of 0–64). Urine Glc4 concentrations were determined using UHPLC MS/MS method.

Results

Our data showed that urine tetrasaccharide level of healthy subjects decreased by aging ($p < .001$, $r = -0.256$). Especially it was high in first year of life compared to older subjects. Tetrasaccharide level of Pompe patients were higher than age-matched healthy controls (99 ± 68 mmol/mol creatinine for Infantile onset vs 4.0 ± 3.0 mmol/mol creatinine for age matched healthy control) and 12.1 ± 17.4 mmol/mol creatinine for Late-onset vs 1.7 ± 1.2 mmol/mol creatinine for age matched healthy control).

Conclusions

The result from this study showed that the reference intervals of tetrasaccharide in urine changed with age therefore definition of the decision levels by age is critically important for diagnosis of late onset Pompe disease.

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W178

Spectrum of mutations in HBB gene among thalassemia major patients in a cohort of Nepalese population

S. Thapa, A.J. Kunwar, N. Thakur, S. Kafle
Kathmandu Center for Genomics and Research Laboratory, Nepal

Background-aim

The thalassemias are the most common monogenic disorders result in the expression of absence or reduction of b-globin gene leading to an imbalance between α and β globin chain. Moreover, it is a heterogeneous inherited disorder in its phenotype, geographical distribution and molecular mechanism.

Methods

DNA was extracted from the clinically diagnosed blood sample after informed consent form. Subsequently, Amplification Refractory Mutation System - Polymerase Chain Reaction (ARMS PCR) was used for amplification to analyze mutations in hbb gene. Consequently, 2% agarose gel electrophoresis was used for visualization of PCR products under UV Trans illuminator.

Results

Among 26 β -thalassemia major patients, 13 (50%) had IVS 1–5 (G > C) mutation, 8 (30.76%) had 619 bp deletion, 2 (7.69%) CD 8/9 (+G), 1 (3.84%) CD 15 (G > A), 1 (3.84%) had –88 (C > T) mutation whereas CD41/42 (-TCTT) was not detected in any of the patients. Among the patients with 619 bp deletions 2 (25%) were homozygotes and 6 (75%) were heterozygotes.

Conclusions

This is the baseline study to assist in the regulation of proper new health policies which will impact in the appropriate diagnosis, screening, drug sensitivity, immunogenic assay and management.

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W179

Hereditary haemochromatosis associated to HFE gene's retrospective study and its possible demand at a university hospital

R.A. Torrado Carrión, T. Pascual Durán, V. Cámara Hernández, V. Benito Zamorano, Y. Douhal, G. Sánchez Helguera
Servicio de Análisis Clínicos y Bioquímica Clínica, Hospital Universitario de Getafe, Getafe, Spain

Background-aim

Hereditary Haemochromatosis type I (HHC) is an autosomic recessive metabolic iron disease due to HFE gene's mutations inheritance and it is prone to impact in Caucasian men. Although mutations C282Y and H63D are the most prevalent, S65C is also possible. It is recommended performing Genetic Testing and Screening in patients showing HHC symptoms or high Ferritin values (>300 mg/dL) and Transferrin Saturation levels > 45%.

The objective was to determine mutations frequency from 2003 to 2017, and analyzed if the demand of a Genetic Testing was based on real needs.

Methods

We carried out a retrospective study searching Clinical and Biochemical information in the archives. We obtained the DNA extraction using the tool MagNA Pure Compact (Roche®) followed of the DNA Detection and Amplification through a real time PCR with the tool LightMix® with the auto-analyser Light Cycler 2.0 (Roche®).

Results

During the last 13 years we analyzed a total of 1890 samples liable to contain HHC. A 58.52% (1106) showed a HFE Gene mutation and, of these, a 55.78% (617) showed a mutation in the heterozygous state as the most frequent. Those related to HHC type I represented an 8.59% (95) of the Doubly Heterozygous C282Y/H63D, a 4.43% (49) of the Homozygous C282Y and a 0.09% (1) of the Doubly Heterozygous C282Y/S65C. We did not find any of these 3 mutations in a 41.48% (784). In addition to Medical Backgrounds and History, we analyzed those demands containing Transferrin Saturation and Ferritin to value if the Biochemical Testing presented concordance with the mentioned mutations. In 1713 cases the Biochemical criteria to apply for a Genetic Testing were not accomplished.

Conclusions

The most common genotype was Heterozygous H63D. Although this does not mean a serious risk of suffering from HHC, testing HFE gene is vital to HHC early diagnostic. A high number of samples that did not accomplish Biochemical criteria were found, because in most ones only Clinical Criteria and Medical Background were taken into account. Therefore, we do consider positive optimizing the demand of Genetic Testings' taking into account the Transferrin Saturation levels and ferritin values due to its high negative predictive value for HHC.

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W180

Inborn errors of metabolism in south Indian population

K. Vaidyanathan^b, D.M. Vasudevan^a
^aDepartment of Biochemistry, Amrita Institute of Medical Science, Kochi, Kerala, India
^bDepartment of Biochemistry, Pushpagiri Institute of Medical Science & Research Center, Tiruvalla, Kerala, India

Background-aim

Inborn errors of metabolism (IEM) comprise a large group rare genetic disorders arising due to mutations in genes encoding metabolic pathways. Even though individually rare, collectively they have a high incidence. This study was done to find out the incidence of various IEM in the South Indian population.

Methods

10,125 patients were screened for different metabolic disorders during the time period September 2006–August 2018. The breakup of tests are as follows; Total number of tests – 10,125, Urine screened for metabolic disorders – 2115, Amino acids by HPLC – 601; Organic acids by HPLC – 420, Homocysteine estimation by ELISA – 1225, VMA estimation by column chromatography – 660, 17 alpha hydroxy progesterone estimation by ELISA (for congenital adrenal

hyperplasia) – 1451, Adenosine deaminase estimation by colorimetric test – 2611, Porphyrins screening – 280, Other tests – 762 (Myoglobin, 5 HIAA, glucose 6 phosphate dehydrogenase, homocystinuria etc). Urine screening panel included tests for aminoacidurias, organic acidurias and disorders of carbohydrate metabolism (galactosemia, glycosuria, fructosuria, pentosuria and mucopolysaccharidoses).

Results

The breakup of positive cases was as follows; Aminoacidurias – 40, organic acidurias – 51, hyperhomocysteinemia – 305, pheochromocytomas and neuroblastomas – 51, elevated adenosine deaminase levels – 386 and congenital adrenal hyperplasia – 342. Specific disorders detected included propionic aciduria (PAA), 16 cases, methylmalonic aciduria (MMA), 15 cases, maple syrup urine disease (MSUD), 16 cases, alkaptonuria, 11 cases, tyrosinemia, 6 cases, nonketotic hyperglycinemia (NKH), 9 cases and phenyl ketonuria (PKU), 3 cases.

Conclusions

The incidence of IEM in South Indian population is high. There is a need for introducing newborn screening in this population for early detection of IEM.

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W181

NewSTEPS: A model for continuous quality improvement in newborn screening programs

E. Darby, S. Edelman

Association of Public Health Laboratories, United States of America

Background-aim

In the United States, newborn screening (NBS) is a state public health service intended to identify individuals in a population at an increased risk of certain heritable disorders. It is recognized as the largest and most successful disease prevention system in the United States. However, as NBS is a state mandated system and programs are governed by state legislation, screening protocols, reporting, and the number of disorders screened varies by state.

The Newborn Screening Technical assistance and Evaluation Program (NewSTEPS) – a program of the Association of Public Health Laboratories – is a national NBS resource center designed to provide data, technical assistance and training to all NBS programs and assist states with quality improvement initiatives. These activities have resulted in sustainable NBS programmatic improvements.

NewSTEPS aims to improve health outcomes for infants affected by NBS conditions by offering training, data collection and analysis, and technical assistance to state NBS programs.

Methods

NewSTEPS has built relationships with NBS stakeholders and created a community of engaged public health professionals by offering national meetings, training opportunities, laboratory site visits and reports, educational webinars, online forums, and a comprehensive resource center with a national NBS data repository. These activities take a systems approach to improving NBS programs.

Results

Since its inception in 2012, NewSTEPS has hosted 10 national meetings, 13 site reviews, 125 archived webinars, and supported 59 participants in hands-on workshops in tandem mass spectrometry. The NewSTEPS website hosts over 320 resources including, but not limited to, data visualizations, model practices, and educational tools. NewSTEPS has cultivated an active online listserv community with over 700 members issuing an average of 30 messages per month. The NewSTEPS Data Repository collects state profile level data, case level data and quality indicator data metrics from all 50 state NBS programs and 3 territories and has over 400 registered users. Analysis from the data repository allows NBS programs to identify gaps in the system and make improvements to their operating procedures.

Conclusions

The success of NewSTEPS can serve as a model for NBS quality improvement practices worldwide.

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W182

Combined biomarker analysis improves the LABORATORY diagnostics of sarcoidosis

M. Fagyas^d, K. Király^d, A. Enyedi^c, I.T. Altörjay^a, B.J. Varga^d, E. Kalina^b, J. Kappelmayer^b, Z. Papp^d, I. Takács^c, A. Tóth^d

^aDepartment of Cardiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

^bDepartment of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

^cDepartment of Surgery, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

^dDivision of Clinical Physiology, Department of Cardiology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary

Background-aim

Sarcoidosis is a systemic granulomatous disease of unknown origin, which mostly affects the lungs, the hilar lymph nodes and the skin. It is difficult to set up the diagnosis of sarcoidosis because there are no definitive (gold standard) laboratory tests and therefore it is often necessary to perform invasive biopsy.

Our aim was to determine and compare the diagnostic accuracy of our optimized ACE activity measurement assay and the commercially available ACE activity and other biomarker measuring kits.

Methods

We enrolled 99 patients into the study, they underwent diagnostic mediastinoscopy and hilar lymph node biopsy. Histopathologic results were compared with biomarker concentrations and activities measured from patients' sera. We also determined reference ranges for the examined biomarkers involving 133 healthy individuals.

Results

Sarcoidosis was confirmed by histopathology in 66 patients out of 99. Our optimized fluorescent kinetic ACE activity measuring assay

proved to have the highest diagnostic accuracy (area under the curve (AUC) of receiver operating characteristic (ROC) curve) = 0.813, $p < .001$) among the examined ACE activity tests (Infinity ACE® AUC = 0.807; ACEcolor® AUC = 0.781). Serum chitotriosidase activity (AUC = 0.865, $p < .01$) and soluble interleukin 2-receptor concentration (AUC = 0.660, $p = .010$) can discriminate between sarcoidotic and control patients, while serum amyloid-A (AUC = 0.557, $p = .363$) and lysozyme (AUC = 0.602, $p = .102$) concentrations can not. Joint determination of serum chitotriosidase activity and ACE activity (AUC = 0.890, $p < .001$) can improve diagnostic accuracy resulting in sensitivity of 90%, specificity of 75%, positive predictive value of 89% and negative predictive value of 78%.

Conclusions

We can diagnose sarcoidosis by our optimized ACE activity and chitotriosidase activity assays more accurately than ever, therefore invasive biopsy can be avoided in majority of sarcoidotic cases.

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W183

Senior-Løken syndrome: About two cases

O. Grunewald^a, I. Fajardy^a, I. Drumare^b, S. Defoort-Dhellemmes^b, C. Dhaenens^a

^aDepartment of Biochemistry and Molecular Biology, UF Génopathies, Lille University Hospital, Lille, France

^bExploration of Vision and Neuro-ophthalmology Department, Lille University Hospital, Lille, France

Background-aim

Senior-Løken syndrome (SLS) is a rare autosomal recessive disease with a prevalence of 1/1,000,000. The main associated features are nephronophthisis and retinopathy, like Leber congenital amaurosis (LCA) or retinitis pigmentosa (RP). A progression to blindness and end-stage renal disease (ESRD) is usual. Up to date, nine genes are known to cause SLS, including NPHP1 to NPHP6. Hereby, we present two cases of SLS with clinical and genetic heterogeneity.

Methods

The first case is a 3 year-old girl for whom the only clinical sign was a LCA since her first months of life. She is the first child of a consanguineous couple. A NGS gene-panel including 156 genes of inherited retinal disorders (IRD) revealed the homozygous non-sense mutation p.(Arg364*) (c.1090C>T) in the IQCB1 (or NPHP5) gene. Both parents were heterozygous. This loss-of-function (LoF) mutation has already been described in SLS young patients with a phenotype of early onset LCA and ESRD in the first decade. Therefore, a nephrologic follow-up has been set up in order to prevent the occurrence of renal disease. Moreover, as the couple wishes a new pregnancy, prenatal testing can now be offered to the family.

Results

The second case is a 58 year-old man presenting RP as first symptom for several years and an ESRD with estimated glomerular filtration rate of 28 mL/min per 1.73 m². Several investigations have

been conducted to explore the retinal disorder for a long time, including a 156 IRD genes panel that did not reveal any disease-causing variation. We therefore performed a second NGS panel including 96 genes of hereditary kidney disease. This second exploration found the homozygous frameshift insertion p.(Arg870Trpfs*20) (c.2598_2607dup) in the NPHP4 gene (not present on the IRD panel). This LoF variation has already been described in an adult patient presenting SLS and hearing loss and is considered “likely pathogenic” according to the American College of Medical Genetics and Genomics 2015 recommendations.

Conclusions

These two cases emphasize the importance of multidisciplinary approach in the molecular diagnosis of syndromic conditions, allowing personal medical survey and proper genetic counseling for patients. The upcoming of extended molecular investigations, such as Whole Exome/Genome Sequencing, may contribute this way.

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W184

Selective screening of Galactosaemia in a tertiary lab, South Africa

T. Satekge, O. Kiabilua, T. Pillay
University of Pretoria, South Africa

Background-aim

Galactosaemia is a carbohydrate metabolic disorders presenting early in infancy, occurring as a result of defective enzymes in galactose metabolism. Galactose-1-phosphate uridyl transferase (GALT) deficiency is a common form of hereditary Galactosaemia resulting in metabolic encephalopathy, hepatic and reproductive complications. Screening of Galactosaemia is crucial in order to diagnose early and promptly restrict dietary galactose to prevent significant morbidity and mortality.

Methods

Data of urinary reducing sugars results in our lab for 5 years (2013–2017) was retrieved. Data of follow-up confirmatory tests of Galactosaemia namely, GALT activity and DNA sequencing of the screened patients was also analyzed.

Results

878 pediatric patients were screened. 52% tested positive on the Benedict's test of which 48% of these had galactose present on thin layer chromatography. 31% of patients with galactosuria were followed up with GALT activity of which 12 patients (38%) of had pathological low GALT activity. Furthermore, 12 more patients who had tested negative on Benedict's test and had no galactosuria present also reported significantly low GALT activity. Only 2 of the screened patients had DNA sequencing.

Conclusions

A sizeable proportion of our population presenting with galactosuria was not followed up with GALT activity. This was likely due to resources constraints and the pre-analytical limitations of the

GALT enzyme. Genetic tests were shown to be under-utilized in confirming Galactosaemia. A notable number of Galactosaemia cases were potentially missed by the screening tests.

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W185

Aberrant mutation spectrum and clinical characteristics of patients with mucopolysaccharidosis type I in an Iranian series

M. Abdi^a, M. Taghikhani^f, S. Khatami^d, M. Alaei^e, D. Zamanfar^c, R. Vakili^b, M. Hakhamaneshi^a

^aKurdistan University of Medical Sciences, Iran

^bMashhad University of Medical Sciences, Iran

^cMazandaran University of Medical Sciences, Iran

^dPasteur Institute of Iran, Iran

^eShahid Beheshti University of Medical Sciences, Iran

^fTarbiat Modares University, Iran

Background-aim

Mucopolysaccharidosis type I (MPSI) is a rare autosomal recessive disorder caused by a deficiency of α -L-iduronidase (IDUA) encoded by the IDUA gene. It is highly important to always determine and update the genotypic information of a population for a specific disease. In Iran, there is a paucity of studies based on a limited number of patients and therefore its utility was confined to the needs at that time. Therefore, in the present study we investigated the mutation spectrum of the IDUA gene to describe the clinical, biochemical and molecular characteristics of an Iranian population with MPSI.

Methods

Mutational screening of the known coding region and exon-intron boundaries was performed by sequencing analysis. We recorded clinical features of studied patients at the first diagnosis of disease and then during the treatment and follow-up. Urinary GAGs was measured calorimetrically based on the reaction of DMB with urinary mucopolysaccharids and results were expressed in mg/g creatinine.

IDUA activity in dried blood spots (DBS) samples was measured fluorometrically and results were expressed as $\mu\text{mol}/\text{spot}\cdot\text{incubation time}$ and low range results were confirmed by leukocyte assay.

Results

Five different missense disease-causing mutations were identified in our patient group; giving a detection rate of 90.48%. The most prevalent mutation was the p.Y109H, occurring in 15.625% of all alleles, which report for the first time in our study. Other frequent mutations were: p.S157P (12.5%), p.G84R (12.5%), p.D257H (9.375%), and p.D301E (9.375%). Of them, three were novel missense mutations: p.S157P, p.D257H and p.D301E. There were not any significant correlation between genotype and related phenotype in the studied subject.

Conclusions

Results of the present study show the different spectrum of IDUA gene mutations in our MPSI patients. In this series, the occurrence of

clinical features had no correlation with genotype variations and biochemical findings.

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W186

Whole-exome sequencing as first-tier testing approach for identification of the causal mutations in hereditary spherocytosis candidate genes and the use of non-sanger-based methods for validation of the findings

N. Camila, T. Santa Rita, P. Mesquita, R. Jácomo, L. Nery, G. Barra
Sabin Medicina Diagnóstica, Brazil

Background-aim

Hereditary spherocytosis (HS) is a common form of inherited hemolytic anemia characterized by hemolysis, jaundice, splenomegaly, and gallstones. The condition commonly exhibits an autosomal dominant pattern of inheritance. Causal mutations in at least five genes have been reported so far (SPTA1, SPTB, ANK1, SLC4A1, and EPB42). In this study, we aimed to investigate the five HS genes performing whole-exome sequencing (WES) to identify the causal mutations in a cohort of subjects with the condition. A secondary aim, we tested if non-sanger-based methods, such as ARMS-qPCR (for SNV) and capillary electrophoresis (for INDELS), can be used to validate the next-generation sequencing findings.

Methods

The studied cohort comprises 16 patients (8 males) with HS diagnosis. There was one pair of siblings, one mother-daughter pair, one mother and two sons, and nine unrelated subjects. DNA was extracted from whole blood using MagNA Pure 96 (Roche), quantitated using Qubit (ThermoFisher), and sized and qualified by gel electrophoresis. Targeted resequencing library was captured using SureSelect All exon V5-post kit (Agilent Technologies). Genomic libraries were quantified and validated using TapeStation (Agilent Technologies) and sequenced as paired-end 150 bp reads on the NextSeq 500 sequencer (Illumina). Sequence reads were aligned to human reference genome 19 using the BWA aligner. Duplicates were removed using Picard and variant calling was performed using the GATK Unified Genotyper (both from Broad Institute). SNV and INDELS were annotated using BaseSpace Variant Interpreter (Illumina). Variant lists were filtered based on having a minor allele frequency of <0.01 on population frequency databases [1000 Genome Project, NHLBI exome sequencing project and exome aggregation consortium (ExAc)]. Mutations that had read depth (coverage) <8 , quality score <20 , or resulted in synonymous amino acid changes were excluded. Sanger sequencing and ARMS-qPCR or capillary electrophoresis were compared for the validation of NGS results.

Results

Pathogenic mutations were identified in 14 out of 16 (87.5%) studied subjects (all in heterozygosis). Six patients had a mutation in the SPTB gene [p.(Arg1423Ter) occurring twice and p.(Gln804Ter) occurring three times, because the subjects were relatives], six patients had a mutation in the ANK1 gene [p.(Arg319Ter), p.(Gln1806Ter), p.(Glu101Ter), p.(Gly122Arg), c.4227+1G>A and c.5518-1G>A], one patient had a mutation in the SPTA1 gene

[p.(Tyr2305Ter)]. All of them have not been previously described and appear to be novel findings. One patient had both SLC4A1 [p.(Trp848Arg)] and SPTA1 [p.(Glu110Val)] mutations, which were found in the ExAc population database at very low frequencies (MAF of 0.000015 and 0.00022, respectively). All identified mutations were confirmed by Sanger sequencing and by ARMS-qPCR or capillary electrophoresis. Two patients (siblings) had no mutation in the five HS genes according to the used filtering criteria and will proceed to all hereditary anemia genes sequence inspection (followed by WES inspection if none causal mutation is found).

Conclusions

The use of whole-exome sequencing as the first-tier testing approach was effective in identifying the causal mutations of HS in the studied cohort (87.5% of the volunteers had the causal mutation identified) and non-sanger-based methods could validate the NGS results.

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W187

Interpretative cut-off for alpha-1-antitrypsin concentration in detection of alpha-1-antitrypsin deficiency among pediatric patients - A pilot study

A. Beletic^a, Z. Lekovic^{d,f}, Z. Zivkovic^{b,e}, N. Radlovic^{d,f}, V. Perisic^{d,f}, M. Ljujic^c, D. Radojkovic^c, V. Djordjevic^c, S. Stankovic^a

^aCenter for Medical Biochemistry, Clinical Center of Serbia, Belgrade, Serbia

^bHospital for Children's Lung Diseases and Tuberculosis, Clinical-Hospital Center "Dr Dragisa Misovic", Belgrade, Serbia

^cInstitute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia

^dUniversity Children's Hospital, Belgrade, Serbia

^eFaculty of Pharmacy, University Business Academy in Novi Sad, Novi Sad, Serbia

^fSchool of Medicine, University of Belgrade, Belgrade, Serbia

Background-aim

Alpha-1-antitrypsin deficiency (AATD) is an autosomal recessive disorder characterized by the reduced alpha-1-antitrypsin (AAT) level in blood. In pediatric patients, it is dominantly tested as a cause of liver disease, while specific lung diseases might be potentially regarded as additional indications. Measurement of AAT concentration is useful as a first-line test, although decreased level may be also encountered in certain acquired conditions. Our aim was to determine the interpretative cut-off for AAT concentration i.e. level below which the presence of common AATD-associated alleles might be suspected.

Methods

We included 37 subjects with clinical indications or familiar history of AATD: 19 males and 18 females, aged between 2 months and 19 years. Immunonephelometry was used for measurement of AAT concentration in serum and PCR-reverse allele specific hybridization assay for detection of Z and S alleles, which are considered as the common AATD-associated alleles. Kruskal-Wallis test and ROC analysis were applied in statistical evaluation.

Results

Three cases of AATD (ZZ genotype) and 14 carriers (13 heterozygous for Z and one for S allele) were detected. AAT concentration (median (min-max)) measured in AATD cases (0.35 (0.31–0.39) g/L), carriers (0.81 (0.56–1.41) g/L) and patients with no Z and S allele (1.20 (0.91–2.24) g/L) were significantly different ($P < .001$). Four carriers (three heterozygous for Z and one for S allele) had AAT concentration in the reference range (0.9–2.0 g/L). We identified the level 1.03 g/L as the most appropriate cut-off to distinguish group comprising both cases of AATD and carriers from patients in whom no common AATD-associated allele was present. Corresponding AUC value (95% Confidence interval (CI)) was 0.929 (0.795–0.987) ($P < .001$). Sensitivity and specificity (95% CI) reached 94.1 (71.3–99.9)% and 90.0 (68.3–98.8)% respectively.

Conclusions

In pediatric patients AAT concentration below 1.03 g/L may be regarded as a potential cut-off indicating the presence of common AATD-associated alleles in homo- or heterozygous form. Nevertheless, this preliminary finding needs confirmation in a larger study.

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W188

False elevation of urinary porphyrins after prolonged sedation with propofol. Analysis of an interference

C. Castillo Perez^b, F.J. Illana Camara^c, M.J. Torrejon Martinez^a

^aHospital Clinico San Carlos, Spain

^bHospital Fundacion Jimenez-Diaz, Spain

^cLaboratori de Referencia de Catalunya, Spain

Background-aim

Porphyries are a group of metabolic diseases caused by deficiency of an enzyme involved in heme synthesis. Certain drugs may precipitate a porphyric crisis. One anesthetic considered safe for using in these patients is Propofol.

However, porphyrin elevation has been described after a prolonged sedation with Propofol in some studies.

Methods

We measured 24 h urinary porphyrin excretion in 4 patients sedated with Propofol at least 24 h. The method used for the porphyrin determination was reverse phase HPLC with fluorescence detection and a column C18 as stationary phase. A follow-up of 48 h was performed in order to evaluate the chromatogram pattern after the propofol withdrawal. Interference studies were performed using @-glucuronidase and sulfatase type H-1 enzymes.

Results

An increase in total urinary porphyrin excretion as well as an increase in hexaporphyrin and uroporphyrin was found after Propofol sedation. Progressive normalization of urinary porphyrins followed anesthetic withdrawal.

Samples treated with @-glucuronidase enzyme showed no interference pattern and a normal total porphyrin, uroporphyrin

and hexaporphyrin excretion. Sulfatase type H-1 enzyme did not remove the abnormal peaks in the chromatogram.

Conclusions

Prolonged Propofol sedation caused a false increase in total urinary porphyrins, uroporphyrin and hexaporphyrin mediated by abnormal peaks caused by the glucuronidation of propofol in liver after a prolonged sedation of at least 24 h. This event should be considered to avoid false positive in porphyrins determination.

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W189

Neonatal hyperammonemia as a consequence of a defect of the urea cycle

R. González, L. González, A.I. Álvarez, A. González, J.M. Guerrero, C. Delgado

Hospital Universitario Virgen del Rocío, Spain

Background-aim

Disorders of the urea cycle are secondary to a defect in the path that converts ammonia into urea, resulting in accumulation of ammonia and other products. Newborn presentation is the most common of these defects and presents with somnolence, poor feeding, hyperventilation, and seizures, followed by lethargy and coma.

Methods

We present the case of a newborn of 41 months and 3 weeks with 48 h of life admitted to the Critical Care Unit (CCU) due to develop seizures and respiratory depression.

Forty-eight hours after discharge of the CCU, the newborn was complaining, with coldness, sweating, rejection of food and tonic-clonic seizure and was administered rectal diazepam. Later, he presented skin pallor, jaundice and generalized hypotonia and he did not response to stimulation.

Results

High levels of lactic acid (11.5 mmol/L, ref.: 0.5–1.5) and ammonia (2025 μ mol/L, ref.: <150) were observed in the analytical results, so additional tests were requested to the laboratory due to the suspicion of a metabolic disease.

The amino acid profile by tandem MS-MS revealed an increase in glutamine levels (1740 μ mol/L, ref.: 371–641), alanine (740 μ mol/L, ref.: 216–443), ornithine (391 μ mol/L, ref.: 43–118), lysine (401 μ mol/L, ref.: 99–209), and undetectable citrulline levels. Thinking of a possible disorder of the urea cycle, the orotic acid in urine is performed as a second tier test. Orotic levels were 792 mmol/mol creatinine (ref: 0.1–3.7). Moreover, samples for a genetic study were also requested and a variant in hemizygous (c.790A>C, p. Thr264Pro) in the ornithine transcarbamylase (OTC) gene was detected. This result did not confirm or exclude the diagnosis because it is a mutation not described in the literature. However, when a highly conserved amino acid is affected, the bioinformatic analysis suggests its pathogenicity, being recommended the study of relatives and of the mother due to the X-linked recessive inheritance of the disorder. When the mother was studied, the same mutation was found and it supported the diagnosis of an OTC deficiency.

Conclusions

- * Neonatal hyperammonemia is a medical emergency that should make us suspect the existence of a metabolic disease. A rapid diagnosis is essential, because it involves a medical emergency with high mortality and serious neurological complications.
- * A suspect diagnosis of the urea cycle disorders is obtained from a characteristic pattern of altered amino acids whereas the genetic study is being done.

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W190

New heterozygous variant in DNAJC5 found in the diagnosis of lipodosis

J. Liró Armenteros^c, M. Fabre Estremera^a, M. De Toro Crespo^c, P. Jimenez-Arriscado^b, E. Calderon^b, J.M. Guerrero Montalvez^b, H.C. Macher Manzano^b

^aH. Clínico Lozano Blesa, Spain

^bH. U. Virgen del Rocío, Spain

^cH. U. Virgen Macarena, Spain

Background-aim

Adult neuronal ceroid lipofuscinoses (ANCLs) are a genetically heterogeneous group of neuronal ceroid lipofuscinoses with onset during the third decade of life, characterized by dementia, seizures and loss of motor capacities, and sometimes associated with visual loss caused by retinal degeneration. These symptoms are similar to those of other lipodosis.

Methods

The laboratory is requested to study enzymatic and genetic study of Gaucher disease in a patient with a quitotridase levels of 1241.3 nmol/ml/h (normal: 4–76 nmol/ml/h).

The patient is 57 years old and has pancytopenia besides other vague symptoms not related. We receive an EDTA blood sample, lymphocytes was separated for enzymatic study and DNA was extracted by the MagNA Pure system (Roche) and has been sequenced with a custom design panel for lysosomal diseases of Ion Ampliseq for Thermo Fisher S5 Ion Torrent Platform.

Results

The β -glucosidase activity was normal 8.1 μ mol/L/h (normal: 2.5–40 μ mol/L/h) and we did not found any pathology in GBA and PSAP exome.

However a variant of uncertain significance was identified in DNAJC5 (NM_025219.2) c.192C>T and p.His64=, not described previously, despite a synonymous amino acid change, "In silico" predictors Mutation T@ster and Human Splicing Finder describe it like a potentially pathological because of an alternative splicing site.

Conclusions

The DNAJC5 gene encodes the cysteine string protein, a presynaptic J protein expressed in neural tissues as well as in synaptic and clathrin-coated vesicles.

DNAJC5 gene is a family member of heat shock proteins DNAJ and encodes J proteins that are expressed in neuronal tissues and

synaptic vesicles. These proteins regulate ATPase activity in many cellular processes. The encoded protein plays an important role in the transport of substances through the membrane and in the folding of other proteins, so they are attributed antineurodegenerative properties.

This process might be particularly critical for the viability of neurons because mutations in DNAJC5 are known to cause ceroid lipofuscinosis in adults with a dominant inheritance pattern.

Rare diseases have crossed symptoms making them difficult to diagnose and no screening method exists, but nowadays massive sequence approach could save a lot of time to these minority diseases.

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W191

Gaucher's disease in a 19-year-old woman: Case report

H. Broutier^b, Z. Hebib^a, R. Sahli^a, C. Petit^b, W. Masri^b, E. Plouvier^b, H. A. Cung^b

^aClinical Hematology, GHEF, Meaux Site, Meaux, France

^bLaboratory, GHEF, Meaux Site, Meaux, France

Background-aim

Gaucher disease (GD) is a rare, autosomal recessive genetic disorder. It is caused by a deficiency of the lysosomal enzyme, glucocerebrosidase (GCase), which leads to an accumulation of its substrate, glucosylceramide, in macrophages. Here, we report a case of a 19-year-old woman who presented a pancytopenia associated with hepatosplenomegaly. There are no other clinical signs or neurological abnormalities.

Methods

NFS was measured with Sysmex XN 3000®. Serology, biochemistry and hemostasis were determined respectively on Cobas 6000® (Roche) and on STAR Max® (Stago). Bone marrow aspiration was performed. GCase level was measured by fluorimetry. A genetic study was realised in Necker Hospital.

Results

The hemogram study revealed pancytopenia: anemia (Hb = 10 g/dL), leucopenia (1.49 G/L), thrombopenia (53 G/L). Liver enzymes were increased slightly to moderately (aspartate aminotransferase = 60 IU, alanine aminotransferase = 48 IU) but serum proteins and albumin, kidney function test and urine analysis were unremarkable. Other laboratory findings disclosed: total bilirubin 40 µmol/L (reference range < 21), direct bilirubin 10 µmol/L (reference range < 5), and prothrombin time activity 58.0% (reference range 70–100). To find out the cause of pancytopenia, we performed serology tests for infection with hepatitis B virus, hepatitis C virus, Epstein-Barr virus and auto-immunity tests; however, there were no remarkable findings. Bone marrow aspiration counts many macrophages, inducing their transformation into Gaucher cells. Under light microscopy, Gaucher cells are typically enlarged, with eccentrically placed nuclei, condensed chromatin and cytoplasm with a heterogeneous crumpled tissue paper appearance.

The serum GCase level was found to be decreased (1.9 U; reference range 6.5–10.5). Results of genetic study will confirm our diagnosis of GD with identification of mutations in the GBA1 gene.

Conclusions

We diagnosed the patient with Gaucher disease type 1, most common form of the disease. It's one of the rare genetic diseases for which therapy is now available. In view of severe pancytopenia, enzyme replacement therapy was the treatment of choice. It will be done in our hospital in coordination with reference center for rare diseases.

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W192

The identify project: Looking for Pompe

D. Rodrigues, P. Crujeiras, J. Cocho, M. Couce, C. Colón
Diagnosis and Treatment of Congenital and Metabolic Diseases Unit, Department of Pediatrics, Hospital Clínico Universitario and Fundación Instituto de Investigación en Salud (FIDIS), Santiago de Compostela, Spain

Background-aim

Pompe disease (PD) is a rare, inherited autosomal recessive storage disorder caused by the deficient activity of lysosomal enzyme acid α -Glucosidase (GAA). It is characterized by a continuum disease spectrum and pending on genetics, it can be classified into two forms: infantile where mutation causes synthesis of a catalytically inactive enzyme protein, and adult form where mutation causes a reduction in the amount of enzyme protein.

The aim of this project is to shorten the time of diagnosis for PD from the appearance of specific signs and symptoms that usually are not taken as possible PD.

For that we started a selective screening strategy in pediatric and adult patients with clinical symptoms compatible with PD covering the whole country (Spain).

Methods

During 2018, we started a national program on a risk population based on clinical criteria.

With the help of scientific societies and pharmaceutical industry we distributed the kits with necessary material: the analytical paper to collect patient's blood, informed consent and a clinical guide with the signs and symptoms to be considered: musculoskeletal (including myalgia and cramps), respiratory (including frequent respiratory infections) and gastroesophageal symptoms (including gastroesophageal reflux). All samples were received by ordinary mail in a mean time of 2 days.

Results

We received a total of 319 samples from patients from Spain. In all of them, the enzymatic GAA activity was measured as main screening method. Beta-Galactosidase (GLB1) activity was also measured as a sample quality control.

After requesting 19 samples in order to confirm the previous result, mass spectrometry was used as a second-tier test, and we were able to detect 4 cases of PD, ranging from 3 months to 61 years.

The positive predictive value of this screening was 21.1% with a precision of 95.3% and a specificity of 95.2%. We do not have information of any false negative.

Conclusions

A based on symptoms screening for PD has shown to be a simple and reliable method for an early diagnosis of PD not detected in usual clinical practice.

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W193

Deletion associated with di George syndrome detected by array-CGH

M. Molina Zayas^d, M.D.M. Del Aguila Garcia^e, S. Garcia Chileme^a, T. De Haro Romero^e, C. Garcia Rabaneda^b, M.J. Aguilar Castillo^e, T. De Haro Muñoz^c

^aComplejo Hospitalario de Jaen, Spain

^bHospital del Mar, Barcelona, Spain

^cHospital San Cecilio, Granada, Spain

^dHospital Universitario de Jerez, Spain

^eHospital Virgen de las Nieves, Granada, Spain

Background-aim

Male patient of three years of age, who has follow-ups by Neuropediatrics. He has a generalized developmental disorder, communication problems -he does not speak, he only emits sounds-he does not make eye contact, he does not pay attention when he is called upon) and he walks on tiptoes.

Methods

In view of these symptoms, doctor requests the cytogenetic study, which consists of:

- Extraction of genomic DNA from a sample of the patient.
- Application of the CGH array technique by the use of a CGX array that includes 180,000 probes designed by Signature Genomics Laboratories, PerkinElmer.
- Analysis of the results using the Genoglyphix software.

The CGH-array technology allows to analyze, quickly and efficiently, losses or gains of genetic material and unbalanced rearrangements throughout the genome. The platform has an average resolution throughout the genome of ~ 20 kb and a resolution of 10 kb in the regions of interest → 250 microdeletion/microduplication syndromes and >250 syndromes associated with autistic spectrum.

The limitation of the technique is that it does not allow to detect balanced chromosomal rearrangements or mosaicisms that appear in a low percentage.

Results

A deletion of 1.33 Megabases in the chromosomal region 22q11.21 has been detected in the patient sample, presenting a male genetic pattern compatible with the formula (ICSN 2009 nomenclature) arr 22q11.21 (18,919,528-20,246,877) x1.

Conclusions

The 22q11.21 deletion is associated with the velocardiofacial/Di George syndrome, however it has a variable phenotype, which in some cases is associated with developmental delay, swallowing

difficulties during childhood, cognitive delay, facial anomalies, autistic spectrum disorder, etc.

Approximately 80% of the mutations are "de novo", in only 5–10% of the cases the deletion is transmitted by the father or mother. A person that carries of the deletion can transmit it to their offspring with a 50% risk. In our case, when the cytogenetic study was performed on the mother, it was observed that she was the carrier of the same deletion and, therefore, the disease was inherited from the mother.

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W194

Implementation of a neonatal program of drepanocytosis screening

B. Prieto^a, Ó. Vega^a, E. Cernuda^a, F. Fernández^a, E. Martínez-Morillo^a, A. Fonseca^b, S. González^b, F.V. Álvarez^a

^aServicio Bioquímica Clínica, AGC Laboratorio de Medicina, Hospital Universitario Central de Asturias, Oviedo, Spain

^bServicio Hematología Clínica, Hospital Universitario Central de Asturias, Oviedo, Spain

Background-aim

In our community, sickle cell screening was implemented in February 2017, completing the Common Basic Portfolio of Services of the National Health System.

In this first year of operation of the protocol, 5622 newborns have been screened.

The aim of this work was to show the first evaluation of the quality indicators of our neonatal program of drepanocytosis screening.

Methods

The screening is performed on the same sample of blood used for the neonatal screening of metabolopathies, obtained by the heel prick test, with the following characteristics:

1. Unification of blood sampling at the corresponding maternity units.
2. A single heel test between 48 and 72 h of the newborn's life.
3. Instrumentation/software: HPLC (VariantTMnbs, BioRad).

Three processes have been evaluated:

- I. Collection and sampling.
- II. Screening (Biochemistry Laboratory).
- III. Diagnostic confirmation and immediate intervention (Pediatric Hematology Unit, PHU).

Results

I. The estimated participation was 99%, with 94% of samples taken before 72 h of life of the newborn and a median age of 52 h of life. There were 124 samples (2.0%) not valid for screening (bad impregnation, insufficient sample ...). Ninety-nine percent of the samples took less than five days to reach the laboratory, with a median of one day.

II. The turnaround time was also less than five days in 99% of samples (median: one day).

The age of the newborn when the report of the first valid sample was sent was <10 days for 99% of cases, with a median of five days. In

those cases in which an abnormal hemoglobin was detected in the initial screening, the heel test was not repeated to verify results, but confirmation was performed by electrophoresis in peripheral blood. One homozygous sickle cell disease and 25 carriers of sickle cell trait were detected, being this incidence higher than the expected a priori in our community. The phenotypes obtained by electrophoretic separation in peripheral blood confirmed all positive screenings as carriers of some hemoglobinopathy in heterozygosis (19 FAS, 4 FAC, 1 FAD, 1 FAE). All detected cases were reported to the PHU on the same day or the next business day.

III. The age at the confirmation/exclusion diagnosis was less than three weeks of age in all cases, according to ministerial recommendations. Given the total agreement between the pattern obtained by HPLC of blood from the heel and the electrophoresis in peripheral blood, after this first year of screening our strategy will be modified to confirm only those affected newborns of sickle cell disease in

peripheral blood. Therefore, the heterozygous carriers will be informed after two different HPLC runs on the first heel prick test sample.

Conclusions

The establishment and evaluation of indicators by process has allowed us to know the effectiveness of the program of neonatal screening for sickle cell disease in its first year of operation in our community. It is considered essential to continue evaluating the program systematically to detect areas of improvement and guarantee the quality of care at all the processes that are part of it.

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