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Mutation of the iron-sulfur cluster assembly IBA57 gene causes lethal myopathy and encephalopathy. *N. Ajit Bolar[1,8], A. V. Vanlander[2,8], C. Wilbrecht[3,8], N. Van der Aa[1], J. Smet[2], B. De Paepe[2], G. Vandeweyer[1], F. Kooy[1], F. Eyskens[4], E. De Latter[2], G. Delanghe[5], P. Govaert[5], J.G. Leroy[2], R. Lill[3,6,7], R. Van Coster[2], L. Van Laer[1], B. Loeys[1].* 1) Department of Medical Genetics, University of Antwerp, Antwerp, Edegem, Antwerp, Belgium, Belgium; 2) Department of Pediatrics, Division of Pediatric Neurology and Metabolism, University Hospital Ghent, 9000 Ghent, Belgium; 3) Institut für Zytobiologie, Philipps-Universität Marburg, Robert-Koch Str. 6, 35032 Marburg, Germany; 4) Provinciaal Centrum voor de Opsporing van Metabole Aandoeningen (PCMA), Department of Pediatrics/Metabolic Diseases, Faculty of Medicine and Health Sciences, Antwerp University Hospital and University of Antwerp, 2000 Antwerp, Belgium; 5) Department of Neonatology, Paola Children's Hospital ZNA Middelheim, 2000 Antwerp, Belgium; 6) Max-Planck-Institut für terrestrische Mikrobiologie, Karl-von-Frisch-Str. 10, 35043 Marburg, Germany; 7) LOEWE Zentrum für Synthetische Mikrobiologie SynMikro, Hans-Meerwein-Str., 35043 Marburg, Germany; 8) The authors wish it to be known that, in their opinion, the first 3 authors should be regarded as joint First Authors.

The iron sulphur [Fe-S] proteins play an important role in redox reactions of the mitochondrial electron transport chain. The de novo synthesis and maturation of these proteins is highly complex and involves more than 25 biogenesis factors. In this study, we have identified two siblings from consanguineous parents who died perinatally from a condition characterised by generalised hypotonia, respiratory insufficiency, anthrogryposis, microcephaly, congenital brain malformations and hyperglycinemia. Analysis of the catalytic activities of the mitochondrial respiratory complexes I and II indicated deficiency in skeletal muscle, suggestive of an inborn error in the mitochondrial iron-sulfur cluster (ISC) biosynthesis pathway. Homozygosity mapping revealed the IBA57 gene, which is known to be involved in the biosynthesis of mitochondrial [4Fe-4S] proteins and present in the largest homozygous region on chromosome 1, as a candidate gene. Mutation analysis of IBA57 identified a c.941 A>C transversion causing the amino acid change p.Gln314Pro. Biochemical analysis of skeletal muscle and skin fibroblasts of affected individuals indicated severely decreased amounts of IBA57 and a decrease in various 4Fe-4S proteins and in proteins covalently linked to lipoic acid. IBA57 depleted HeLa cells reflected biochemical defects consistent with observations in patient derived cells. Defects could be rescued by the introduction of wildtype IBA57 and partially by mutant IBA57. Further functional analysis revealed an increased sensitivity of mutant IBA57 to degradation via proteolysis. Our findings suggest that the mutation leads to functional impairment and degradation below physiologically critical levels, resulting in the condition observed in the patients. In conclusion, we have identified a novel metabolic disorder presenting with a lethal complex biochemical phenotype caused by defective assembly of the ISC protein, IBA57.

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Genetic variation in a gene involved in glycosphingolipid biosynthesis. *O.M. Amaral, A.J. Duarte, E. Pinto, I. Ribeiro, L. Lopes, D. Ribeiro.* Department of Human Genetics, INSA, IP , 4000-Porto, Portugal.

The main objective of this work was to investigate the possible existence of genetic variation in the UGCG gene. The UGCG gene encodes an enzyme essential in the first step of the glycosphingolipid biosynthesis process. Its genetic variation could lead to differences in biosynthesis and be related to phenotypic divergence in various genetic diseases of the glycosphingolipidoses group. In order to test this hypothesis we attempted to identify the extent of variation in the UGCG gene in order to relate it to phenotypic variation. Methods and samples: DNA was extracted from blood samples and/or fibroblast cell lines using an automated apparatus. Biological samples were obtained from healthy donors, with informed consent. In addition, all traceable identification was removed, so as to guarantee their anonymous nature. Skin fibroblast cell lines were obtained from the Corriel Institute (USA). The UGCG gene (exons and flanking intronic regions) of six control individuals was sequenced using standard methods. Results: In this work we present the identification and distribution of genetic variations among the control samples studied. The results obtained with the different samples showed the existence of several polymorphic changes. Discussion: Polymorphisms in the UGCG gene may interfere with the amount of substrate available for degradation in specific diseases along the same pathway. Thus, the degree of genetic variability might influence the phenotypic expression as well as the lysosomal burden. Conclusion: Assessment of variation in the UGCG gene should be considered, particularly in patients who do not comply with the expected genotype/phenotype correlations. Additional information: This work was carried out with financial support obtained from FCT-Portugal: project PIC/IC/82822/2007(2009); AJD and DR were beneficiaries of BI grants from Fundação da Ciência e Tecnologia (FCT/MCTES) - Portugal. Corresponding author: Olga Amaral, olga.amaral@insa.min-saude.pt.

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A zebrafish model of cblC disease displays growth retardation that improves with vitamin B12 therapy. *N.P. Achilly[1], J.L. Sloan[1], K.S. Bishop[1], M.S. Jones[1], V.J. Hoffman[2], R.B. Sood[1], C.P. Venditti[1].* 1) Human Genome Research Institute, National Institutes of Health, Bethesda, MD; 2) Office of the Director, National Institutes of Health, Bethesda, MD.

Cobalamin C disease (*cblC*) is the most common inborn error of intracellular cobalamin metabolism. It is caused by mutations in *MMACHC*, a gene responsible for processing and trafficking intracellular cobalamin. Defects in this pathway impair the function of two cobalamin-dependent enzymes: methylmalonyl-CoA mutase and methionine synthase. Disease manifestations can include growth failure, anemia, congenital microcephaly, heart defects, and progressive blindness. At present, the pathological basis of these symptoms remains unknown, and no animal model exists. To replicate clinical manifestations experienced by patients with *cblC* disease, we created a series of loss of function alleles in the zebrafish orthologue of *MMACHC* using zinc-finger nucleases. Of these, we chose p.L44PfsX21 (hg12) and p.G32VfsX48 (hg13), transmitted by two independent founders, for phenotype analysis. F2 *mmachc*$^{hg12/hg12}$ and *mmachc*$^{hg13/hg13}$ fish survived the embryonic period but displayed growth impairment after 14 days post-fertilization (dpf). By 21 dpf, the standard length (SL) and height at the anterior of the anal fin (HAA) were significantly reduced; *mmachc*$^{hg12/hg12}$ fish (SL 6.94 ± 0.07, HAA 0.77 ± 0.03 mm) and *mmachc*$^{hg13/hg13}$ (SL 7.40 ± 0.07, HAA 0.86 ± 0.01 mm) fish were smaller than the wild-type and heterozygous fish (SL 10.39 ± 0.18, HAA 1.48 ± 0.03 mm) ($p<0.0001$). Histological examination of *mmachc*$^{hg12/hg12}$ fish revealed a complete absence of the secondary lamellae in the gills, which contain specialized cells for gas and ion exchange. Thinner retinal layers and a possible defect in the morphology of the photoreceptor outer segments were also observed. The concentration of methylmalonic acid (MMA), a classic biomarker of *cblC* disease, was elevated by 289-fold in *mmachc*$^{hg12/hg12}$ fish. OH-cobalamin (OH-cbl) injections are the main treatment administered to the patients and ameliorate some of the disease-related complications. When *mmachc*$^{hg12/hg12}$ fish were maintained in water supplemented with OH-cbl (100 µg/ml) for 21 days, SL increased by 25% ($p<0.05$) and HAA increased by 30% ($p<0.01$) compared to the untreated group. The zebrafish model of *cblC* disease we generated recapitulates several of the phenotypic and biochemical features of *MMACHC* deficiency, demonstrates a response to conventional therapy, and should be useful to delineate the pathophysiological mechanisms in this common disorder of cobalamin metabolism.

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Identification of a novel mutation in the human ARSB gene on chromosome 5q14.1 for Muchopolysacharidosis type VI patients in southwest Colombia. *M.A. Acosta Aragón[1], J.R Lago[2], F. Barros[2], A.M. Carracedo Alvarez[2-3].* 1) Pediatrics Department, College of Medicine, University of Cauca, Popayán, Cauca, Colombia, Ph.D; 2) Clinical Hospital of Santiago de Compostela, Galicia, Spain M.D; 3) Institute of Legal Medicine, College of Medicine, University of Santiago de Compostela, Galicia, Ph.D.

Introduction: The MPS VI or Maroteaux-Lamy Syndrome is a recessive multisystemic progressive lysosomic storage disease caused by a deficiency of N-acetylgalactosamine 4-sulfatase enzyme or Arylsulfatase B. A total of 32 patients MPS VI patients are identified in Colombia, sixteen in Cauca Department (southwest Colombia) corresponding to 50% of the total cases with Maroteaux-Lamy syndrome registered in the country. All sixteen patients were identified clinically and by enzyme assay. Two of these individuals with severe form of disease belong to an Amerindian reservation(Guambiano ethnicity). Objetive: to analyze the genomic variations in the Arysulfatase B gene in two patients with severe phenotype of disease and identify ethnic and family backgrounds of the MPS VI Patients in Cauca Department. Subjects: We studied two native patients and their relatives.Data was obtained from charts and families of patients including ethnic and family backgrounds. Methods:It was PCR/sequencing of the 8 exons and their flanking regions in Arysulfatase B gene.We accomplished the validation of exonic changes by computational methods (Alamut 2,HGVS 2). We used 20 single nucleotide polymorphisms (SNPs) for haplotype characterization with a Sequenom Mass Array analyzer. Results: We characterized both alleles in the patients and their relatives identifying a novel mutation p.Ser403X no reported before, both in homozygous or heterozygous form.In addition we identified the same haplotype in the two homozygous patients and their heterozygous relatives when analized this gene with intragenic SNPs.These results together with the genealogy analysis, strongly suggest an inbreeding effect in this population. Conclusions: A novel mutation in the human ARSB gene was reported. It produces a premature stop codon. These results emphasize the broad molecular heterogeneity of Maroteaux-Lamy syndrome and contribute to the establishment of a genotype/phenotype correlation in this disease. The high frequency of MPS VI patients in the Cauca Department and the ethnic characteristics are suggestive that a population genetic factor can be responsible,such as in this study an isolated population with strong inbreeding and consanguinity.Studies with another families of this region with molecular characterization of the mutation by sequencing and the phylogeographic identification of the origin and dispersion of the gene will be performed to clarify this ethnic prevalence.