



“Double Hit” Homozygous Mutations for Two Different Rare Inborn Errors of Metabolism: A Burden for Countries with High Prevalences of Consanguineous Marriages

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ABSTRACT

Inborn errors of metabolism comprise a broad range of genetic diseases of which most are inherited in an autosomal recessive manner. Although being rare, there is a significant increase in their rate especially in countries where consanguineous marriages are performed. Isovaleric aciduria is an organic aciduria characterized by abnormal leucine metabolism resulting from a deficiency in the enzyme isovaleryl-CoA dehydrogenase. Niemann-Pick disease Type C is a rare autosomal recessive inherited disorder involving the intracellular transport of endocytosed cholesterol with sequestration of unesterified cholesterol in lysosomes and late endosomes. Both of these disorders are rarely encountered inborn errors of metabolism. We report a case of a boy with marked jaundice and hepatosplenomegaly, who was later diagnosed as isovaleric aciduria and Niemann-Pick disease Type C concomitantly. The diagnoses were proven by genetic analyses. A novel mutation for Niemann-Pick Type C has also been defined in this case report.

Keywords: Inborn errors of metabolism, Niemann-Pick Type C, isovaleric aciduria, consanguinity

Introduction

Inborn errors of metabolism (IEM) are a group of genetically inherited disorders involving the effects of enzymes of the body on metabolism. Although being rare, there is a significant increase in their rate, especially in countries where consanguineous marriages are performed (1). We report a case of a 2-month old boy who was diagnosed as Isovaleric aciduria and Niemann-Pick disease Type C (NP-C) concomitantly.

Case report

Two-month old boy was admitted due to jaundice and marked hepatosplenomegaly. He was the 3rd child of

consanguineous parents (first degree cousins). His prenatal, natal and family histories were uncomplicated. Jaundice had started when he was 5 days old and was still significant at the time of admission. Hepatosplenomegaly was detected in the outpatient clinic 8th during the routine second month examination and he was referred to our center for further evaluation. His body weight was 5.2 kg (25th percentile) and height was 60 cm (50th percentile). Physical examination revealed yellowing of the skin and eyes, hepatomegaly [4 cm palpable below the lower costal margin (LCM)] and splenomegaly (8 cm palpable below the LCM). Neurological examination showed normal findings. Complete blood count and peripheral blood smear showed normal results. Liver function tests revealed increased γ -glutamyl transferase (277

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U/L), alkaline phosphatase (473 U/L), conjugated bilirubin (5.25 mg/dL), serum glutamate pyruvate transaminase (SGPT) 64 U/L, serum glutamate oxaloacetate transaminase (SGOT) 264 U/L, with normal albumin and International Normalized Ratio (INR). Arterial blood gas analysis, serum lactate and ammonia were within normal limits. Abdominal ultrasonography showed hepatosplenomegaly. He was further evaluated by immunoglobulins, creatine kinase, amylase, thyroid function tests, alfa 1-antitripsine, ceruloplasmine, urine analysis and Benedict's test of urine which showed normal results. TORCH and hepatitis serology were negative. Chitotriosidase (165, normal range 0-120) and alpha feto protein (191.029 ng/mL, normal range 0-7) were elevated. Tandem mass spectrometric analysis using dried blood spots indicated an elevated isovaleryl carnitine level (4 $\mu\text{mol/L}$ [not reported (NR) <0.6 $\mu\text{mol/L}$]. Free carnitine level was within normal limits. His urine organic acid profile showed a markedly increased concentration of isovalerylglycine [80 mmol/mol creatinine (Cr), normal value: 0], and a normal concentration of 3-hydroxyisovaleric acid (6.5 mmol/mol Cr; NR: 0.7-14.4 mmol/mol Cr). In respect to the results of the metabolic tests, genetic testing with Next Generation Sequencing (Miseq-Illumina) for the *IVD* gene was performed using genomic DNA which was isolated from his peripheral leukocytes due to a suspicion of isovaleric acidemia (IVA). A previously reported p.A314V (c.941C>T) homozygous mutation was detected (Figure 1). Although a diagnosis was reached, the patient's splenomegaly still could not be explained and further investigation was essential. With this aim, bone marrow biopsy was performed which

revealed macrophages with abnormal cholesterol storage (Figure 2). Lysosomal enzyme studies from leukocytes were carried out for glucocerebrosidase, sphingomyelinase and acid lipase enzymes which were 5.93 nmol/h/mg protein (normal range 9.4 ± 3.2), 5.13 nmol/h/mg protein (normal range 7.73 ± 3.08) and 150 nmol/h/mg protein (normal range 62.47 ± 35.9) respectively. Since these results made the condition unlikely to be as a result of Gaucher, Niemann-Pick disease Type A-B and Wolman diseases, further studies were performed including Next Generation Sequencing

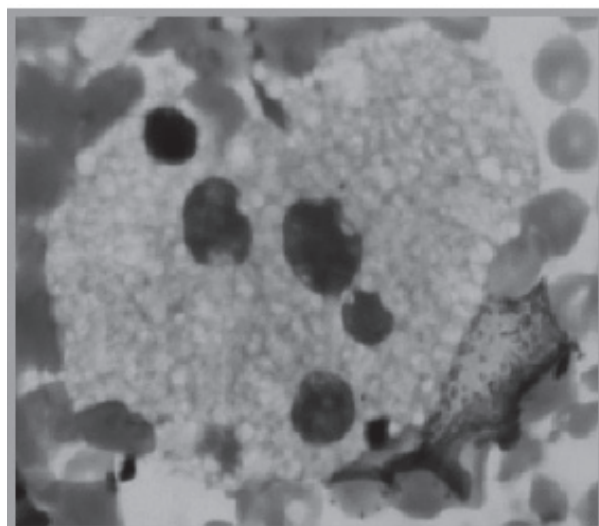


Figure 2. Bone marrow biopsy showing macrophages with abnormal cholesterol storage

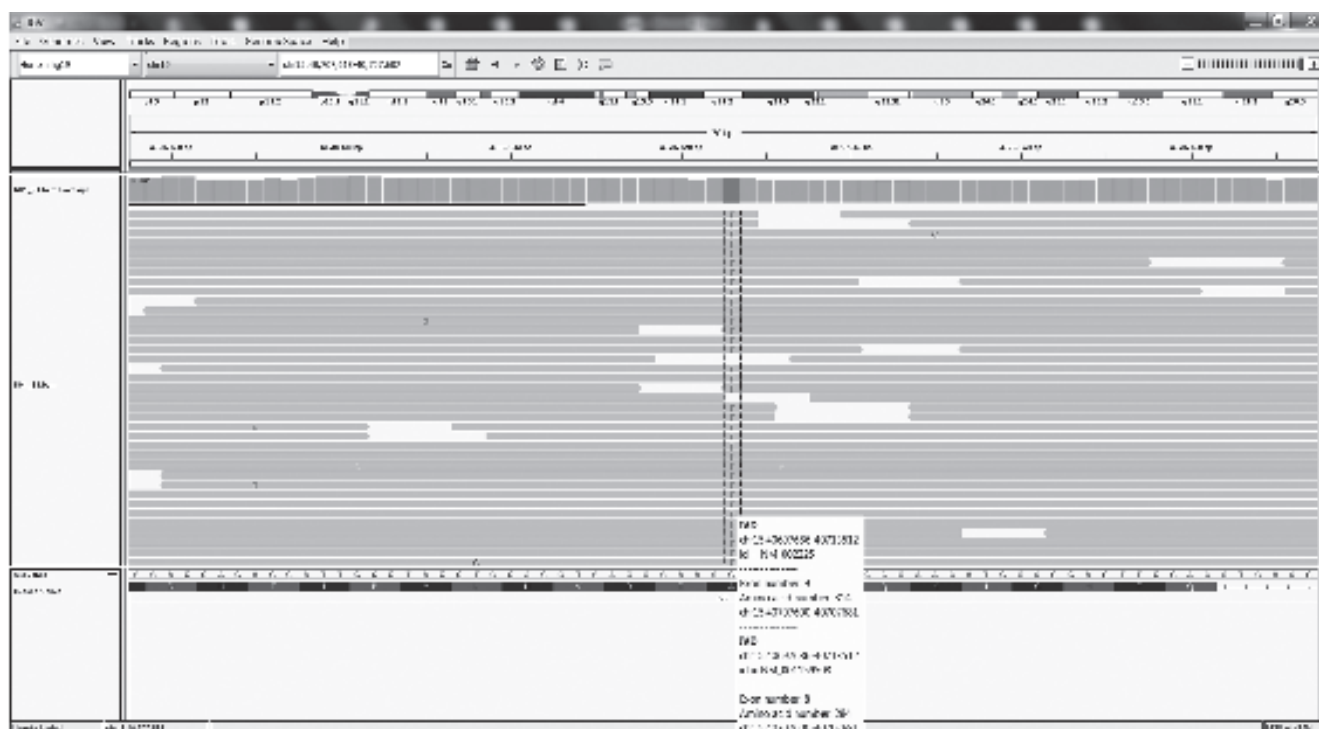


Figure 1. Sequence analysis for *IVD* gene

of *NPC1* gene, which was carried out on DNA isolated from leukocytes, for NP-C. A novel homozygous frameshift mutation in *NPC1* gene: p.P733Sfs*10 (c.2196_2197insT) was detected (Figure 3). Both of the boy's parents were shown to carry both mutations. The patient had remained in a stable condition on glycine supplementation and low-leucine diet. After the age of 1, mild psychomotor retardation, vertical supranuclear gaze palsy and mild spasticity were detected upon physical examination. Electroencephalogram showed irregular background activity with focal slowing. Miglustat therapy was initiated. His neurological condition remained stable after miglustat therapy. He died suddenly at home during an episode of aspiration pneumonia at the age of 2.

Discussion

IEM comprise a broad range of genetic diseases of which most are inherited in an autosomal recessive manner. Individual IEM are rare, most having an incidence of less than 1 per 100.000 births, increasing with the prevalence of consanguineous marriages (2). The incidence of two different IEM occurring concomitantly in a patient has been rarely reported in literature. Guy et al. (3) reported Fabry disease and aspartylglucosaminuria in a patient with consanguineous parents. The authors emphasized consanguinity as a factor in increasing the risk of autosomal recessive disorders occurring concomitantly (3). Concolino et al. (4) reported phenylketonuria and Fabry disease to co-exist in a 3-year old boy with consanguineous parents. IVA is a type of organic aciduria characterized by an abnormal leucine metabolism

resulting from the deficiency in the enzyme isovaleryl-CoA dehydrogenase. The incidence is estimated to be 1 in 62.500-250.000 (5). Currently, there are no published data about the incidence of IVA for Turkey. To date, more than 40 heterogeneous mutations in the *IVD* gene (Online Mendelian Inheritance in Man: 607036), located on chromosome 15q14-15 (<http://www.hgmd.org/>) have been reported. Missense and splicing mutations are the most common. The p.A314V (c.941C>T) homozygous mutation in the *IVD* gene of our patient is a common frameshift mutation and has been previously reported with 20% residual enzyme activity (6). Although it is mostly accepted to cause a mild, asymptomatic clinic with normal development, it is still uncertain whether individuals have a risk of clinical manifestation. While these individuals may have normal leucine homeostasis under physiological conditions, their risk of metabolic decompensation under stress conditions remains to be determined (5). Nasser et al. (7) showed that the A314V (Also reported as A282V) mutant enzyme had thermal instability, a finding that might play a role in the development of symptoms during times of illness. In our patient, initially, a low leucine diet was introduced with a further plan of transforming into a normal protein diet in further follow-up, since the risk of acute decompensation of IVA could not be ruled out completely, as it has been reported in the literature. The low leucine was continued until he was one year old. None of his febrile attacks had been accompanied by metabolic decompensation during this period. NP-C is a rare disorder of intracellular transport of endocytosed cholesterol with sequestration of unesterified cholesterol in lysosomes

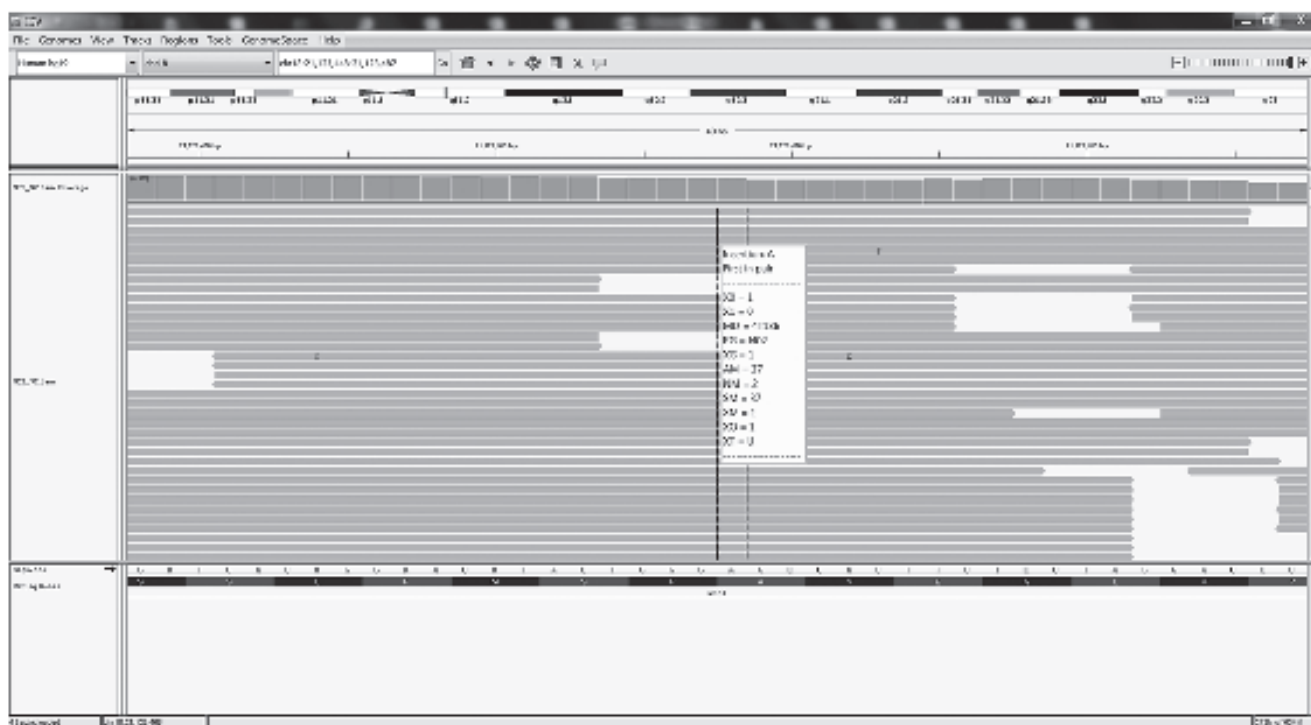


Figure 3. Sequence analysis for *NPC1* gene

and late endosomes. Most of its patients (95%) have mutations in the *NPC1* gene (8). The prevalence in Western Europe has been estimated to be approximately 0.0008-0.0007% of live births. Again, there are no specific data for Turkey. To date, over 300 disease-causing mutations have been reported, most being missense mutations (9). Different clinical phenotypes of the disease can be categorized according to the patient's age at onset of signs. Perinatal presentation is characterized by a prolonged neonatal cholestatic icterus and progressive hepatosplenomegaly, appearing in the first days of life, usually associated with fetal hydrops or fetal ascites. Early-infantile type is characterized by hepatosplenomegaly with neurological findings occurring between 2-24 months. Prolonged neonatal cholestatic icterus associated with progressive hepatosplenomegaly is the most common sign with spontaneous resolution of the icterus by 2-4 months of age. Main neurological symptoms are ataxia, vertical supranuclear gaze palsy (which is considered highly characteristic), dysarthria, dysphagia, dystonia, seizures, cataplexy and progressive dementia (8-10). As reported in the literature, our patient's findings (prolonged jaundice and hepatosplenomegaly which showed up in the second month of life) suggest that he had the early-infantile type of the disease. Neurological signs including psychomotor delay, focal seizures and mild spasticity appeared after 12 months. The mutation of *NPC1* gene described in this study p.P733Sfs*10 (c.2196_2197insT) is a novel frameshift mutation leading to a stop codon (<http://www.hgmd.org/>). As the result is a truncated protein, this mutation most probably causes severe damage to its function. Wild Type protein has 1278 amino acids, while this truncated protein has just 743 amino acids. The disease has been reported with proteins with larger amino acid content. The disease management may include drugs and supportive therapy. Miglustat (ZavescaW, Actelion Pharmaceuticals Ltd, Switzerland), is a reversible inhibitor of glucosylceramide synthase, that has been shown to be effective in the treatment of progressive neurological manifestations in particularly among those with Late-infantile or Juvenile-onset disease, and has been used in that indication in Europe since 2010. It can be initiated as soon as neurological conditions occur. New therapies are in development based on experimental data on substrate reduction therapies (10). In our patient, no seizures were observed after the initiation of miglustat. Although he remained stable, sudden cardiac arrest occurred at home. The exact cause of death could not be identified and an autopsy was recommended which the family refused. Laboratory data which were obtained a few days before his death, in an outpatient clinic showed elevated acute phase reactants, and normal blood gas and ammonia levels. Unfortunately, test to identify his metabolic state regarding isovaleric aciduria could not be performed.

When the clinical picture in a patient cannot be explained by the presence of one rare metabolic disease, it is essential to investigate further for the presence of other IEM's and more attention should be given in individuals with

consanguineous parents. We also would like to emphasize the importance of NBS in the early detection of rare metabolic diseases and more care should be given for the extension of NBS programmes, especially in developing countries. A novel mutation for NPC has also been described.

Ethics

Informed Consent: Has been obtained.

Peer-review: Internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.O., L.T., S.C., G.B., A.H., Concept: A.O., L.T., S.C., G.B., A.H., Design: A.O., L.T., S.C., G.B., A.H., Data Collection or Processing: A.O., L.T., S.C., G.B., A.H., Analysis or Interpretation: A.O., L.T., S.C., G.B., A.H., Literature Search: A.O., L.T., S.C., G.B., A.H., Writing: A.O., L.T., S.C., G.B., A.H.

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