Alpha-mannosidosis and mutational analysis in a Turkish patient

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We present a case of alpha-mannosidosis with its mutational analysis. She was referred to our hospital with the provisional diagnosis of mucolipidosis. She was the first child of second-degree relative parents. She had a coarse face with flat and wide nasal bridge, hepatosplenomegaly, umbilical hernia, lumbar gibbus, motor and mental retardation and deafness. On peripheral blood smear, lymphocytes revealed vacuoles and neutrophils contained some granules resembling Reilly bodies seen in mucopolysaccharidosis (MPS). Based on these findings, the diagnosis of alpha-mannosidosis was suspected. Her urine oligosaccharide chromatography showed an abnormal pattern with a heavy trisaccharide band. Enzyme studies on white cells confirmed a deficiency of alpha-mannosidase activity, which was 2.6 umol/g/hr. Her DNA analysis showed a S453Y mutation.

Key words: alpha-mannosidosis, mutation, neutrophilic granules.

Alpha-mannodisosis is an autosomal recessive lysosomal storage disease caused by deficiency of the enzyme acid mannosidase. Patients affected by alpha-mannosidosis excrete increased amounts of mannose-rich oligosaccharides in the urine, and mannose-rich compounds accumulate in the various tissues of alpha-mannosidosis patients[1]. The severe infantile phenotype is referred to as 'type 1' and a milder juvenile adult type is referred to as 'type 2'. All patients have psychomotor retardation, facial coarsening and some degree of dysostosis multiplex. Common findings include recurrent bacterial infections, deafness, hepatomegaly, hernias, and lenticular or corneal opacities[2]. The clinical features vary in patients and several alpha-mannosidosis causing mutations have been found, but no correlation between the types of mutations and the clinical manifestations could be shown[3]. In this report we present a case with alpha-mannosidosis homozygous for a S453 mutation.

Case Report

A 40-month-old girl was admitted to Hacettepe University İhsan Doğramacı Children's Hospital with developmental delay. When she was six months old her parents became aware of her developmental delay when she could not hold her head, did not follow an object, and did not recognize or react to parents when called. When she was eight months old she was able to hold her head. She could sit with support at 12 months and sit on her own at 24 months of age. She started to walk with the support of her parents a year ago but still could not walk properly. She had acquired only a few words since the previous year. She had several episodes of respiratory infections. She was born as the first child of second-degree relative parents with a body weight of 2700 g 20 days before term. There was no similar patient in the family. The parents applied to another university hospital last year and she was evaluated for mucopolysaccharidosis (MPS). Her magnetic resonance imaging (MRI) showed a delay in myelinization and she was referred to our hospital with a provisional diagnosis of mucolipidosis.

On her physical examination, she weighed 16,500 g (75-90 p), measured 90 cm in length (3-10 p) and 49.2 cm in head circumference. She had a coarse facial appearance, flat and wide nasal bridge, strabismus and epicanthus bilaterally (Fig. 1). She had mild pectus carinatus and umbilical hernia. Liver was palpable 5 cm below the right costal margin and spleen was palpable 8 cm below the left costal margin. She had lumbar

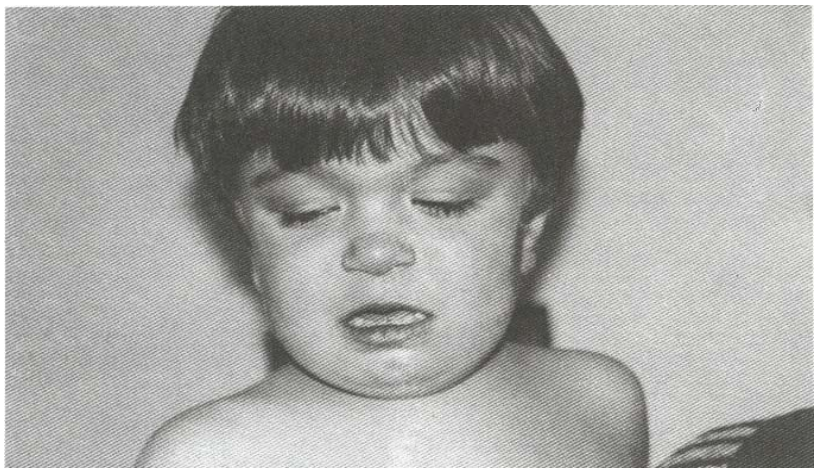

Fig. 1. Facial appearance of the patient with coarsening, flat and wide nasal bridge, strabismus and epicanthus.

gibbus. Her wrists were slightly enlarged. She could not walk without support, she did not talk and she did not obey verbal orders. There was no ataxia or nystagmus. The other physical examination findings were normal.

In complete blood count, hemoglobin was 9.1 g/dl, mean corpuscular volume was 54.9 fl, and red cell distribution width was 18.5. On peripheral blood smear there were vacuoles on almost all lymphocytes (Fig. 2). Most of the monocytes also contained vacuoles. There were cytoplasmic granules on the other unvacuolated lymphocytes. Neutrophils contained some granules resembling Reilly bodies seen in MPS and thrombocytes were cloudy and granular (Fig. 3). Skeletal X-ray findings showed mild dysostosis multiplex with anterior beaking on L2 vertebra, and spatula costa on lung X-ray. Based on her physical examination, X-ray and peripheral blood smear findings, to rule out

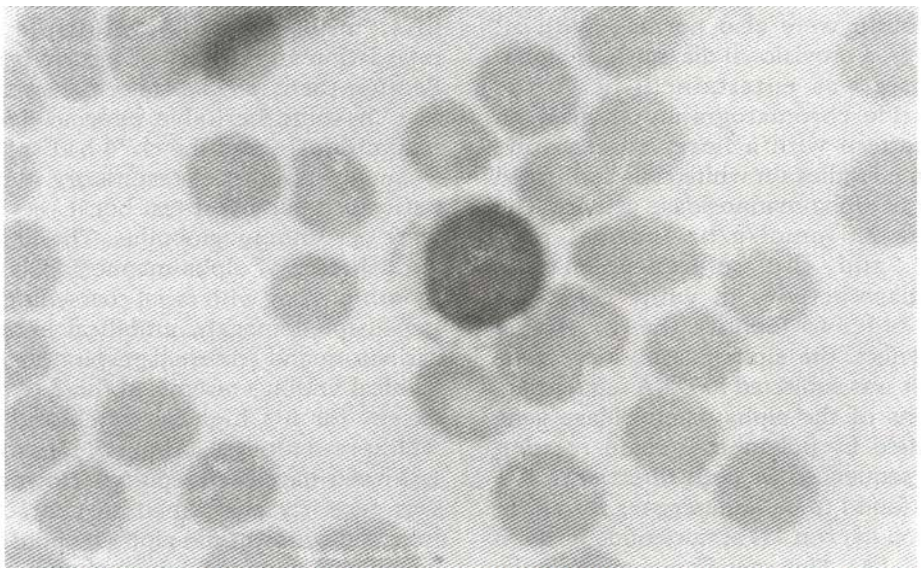

Fig. 2: Vacuoles of the lymphocytes on peripheral blood smear.

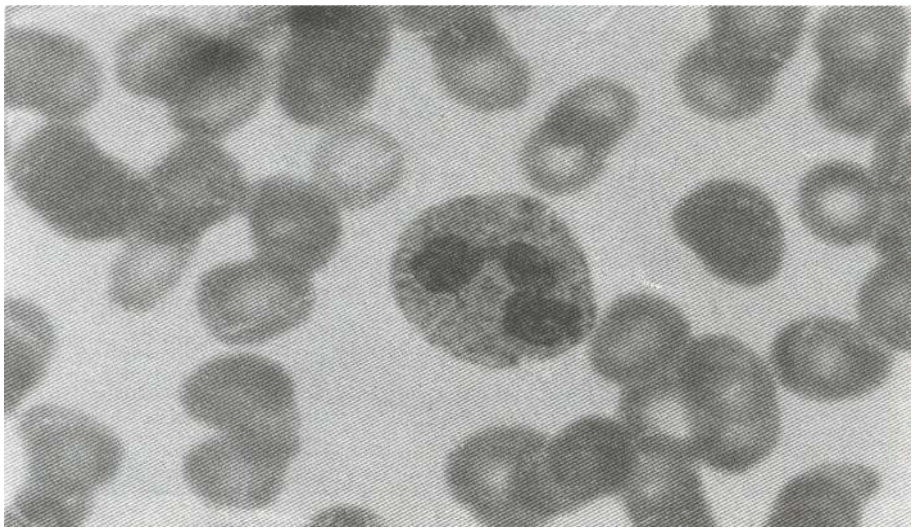

Fig. 3: Neutrophilic granules resembling Reilly bodies.

MPS, urinary MPS analysis was performed and it was normal. Her old MRI findings were reported in our hospital as delay in myelinization that was not specific for any disease. Her audiologic examination showed moderate sensory neural hearing loss by CAVR test. Auditory brainstem response (ABR) could not be performed because of her agitation, but the ABR test in the other hospital showed also retrocochlear hearing loss. Because of recurrent respiratory infections a sweat chloride test was done, and it was found normal. Quantitative immunoglobulin were also normal. With all these findings, a provisional diagnosis of alpha-mannosidosis was entertained; her urine oligosaccharide chromatography showed an abnormal pattern with a heavy trisaccharide band. Enzyme studies on white cells confirmed a deficiency of alpha-mannosidase activity. The activity was 2.6 umol/g/hr (normal: 100-800 units) and the reference enzyme β-galactosidase activity in white cells was normal. In order to identify the underlying molecular defect, the alpha-mannosidase gene of the patient was subjected to DNA sequencing. The 24 exons of the alpha-mannosidase gene were amplified by polymerase chain reaction (PCR) and sequenced in both directions. The patient was found to be homozygous for a C to A transversion in exon 11, at nucleotide position 1358 (1348C→A). This specific mutation results in the replacement of serine with tyrosine at amino acid position 453 in the 1011 residue long alpha-mannosidase polypeptide. As expected, both parents were found to be heterozygous for this particular mutation (1358C→A/+).

Discussion

Alpha-mannosidosis is a rare disorder caused by deficiency of the enzyme acid mannosidase. The clinical manifestations are different in type 1 and 2 diseases and genetic variability is common[1,2].

Our patient had moderate sensorineural hearing loss, mild dysostosis multiplex, mental retardation and history of recurrent infections. Because there may be abnormal sweat chloride test in some heritable enzyme defects[3] like mannosidosis, and the child had suffered from frequent episodes of respiratory infections we performed her sweat test but it was normal, as were her immunoglobulins. The first reported Turkish case of alpha-mannosidosis was a 10-month-old boy with facial coarsening, deafness, hepatosplenomegaly, umbilical hernia, pectus carinatum and recurrent respiratory infections. Clinical findings resembled those of our patient except for his high sweat chloride test and widespread Mongolian spots[4]. Decreased serum IgG levels have been reported. However, a study was recently performed to characterize the immune deficiency in alpha-mannosidosis, and no difference in the levels of immunoglobulin main classes as well as IgG subclasses between

the serum of six alpha-mannosidosis patients and six control healthy subjects was found. On the other hand, it was determined that patients have immune deficiency at both humoral and cellular levels in addition to the immunoglobulin level[5].

On peripheral blood smear, there were vacuoles in lymphocytes and monocytes, cytoplasmic granules were visible in lymphocytes, and neutrophils contained some granules resembling Reilly bodies. In a study done in cats affected with alpha-mannosidosis and MPS VI, by Alroy and colleagues[6], it was shown that vacuoles in lymphocytes is a usual finding in both MPS and alpha-mannosidosis, but cytoplasmic granules in neutrophils was seen only in MPS VI cat, whereas the cytoplasm of neutrophils in alpha-mannosidosis cats appeared foamy, but did not contain cytoplasmic granules[6]. In both of the conditions many platelets appeared vacuolated and hypogranular. These findings on peripheral blood smear deserve to be examined in much more detail in human patients too since more attention is usually given to the vacuoles of the lymphocytes.

Magnetic resonance imaging findings in three adult patients showed brachycephaly, thick calvaria, verticalization of the chiasmatic sulcus, partially empty sella turcica, cerebellar atrophy and white matter signal modifications[7]. Our patient showed only delay in myelinization. Perhaps it is too early to have these changes, the patient might be examined at older ages for MRI changes. In European patients with alpha-mannosidosis, a total of 21 different mutations have been reported, of which the most prevalent mutation, R750W, had previously been identified in a Turkish family[2]. The alteration in the presented case (S453Y), however, has not previously been reported.

The pathogenicity of the S453Y alteration is indicated by the fact that the underlying mutation, 1358C→A, was the only alteration identified in the patient. Moreover, the 1358A allele was absent in 200 Norwegian normal chromosomes (Turkish normal chromosomes were unfortunately not available for this study).

Amino acid residue S453 is conserved in the lysosomal alpha-mannosidases from man, pig, cow and cat. Apparently, however, this residue can be replaced with amino acids containing small or charged side chains such as ala (A), as seen in the mouse, and glu (E), as seen in slime mold (Dictyostelium discoideum). Based on their different biochemical properties the severe consequences of the S453Y alteration can be envisaged. Whereas ser (S) has a small, polar side chain that can hydrogen bond with other residues, tyr (Y) has a large, bulky, aromatic side group that tends to pack in the interior of proteins. Thus, the amino acid replacement represented by S453Y might result in disruption of important hydrogen bonds, distortion of correct folding and, hence, loss of enzyme activity. However, further experiments are required to determine the exact pathogenic mechanism of 453Y (Fig. 4).

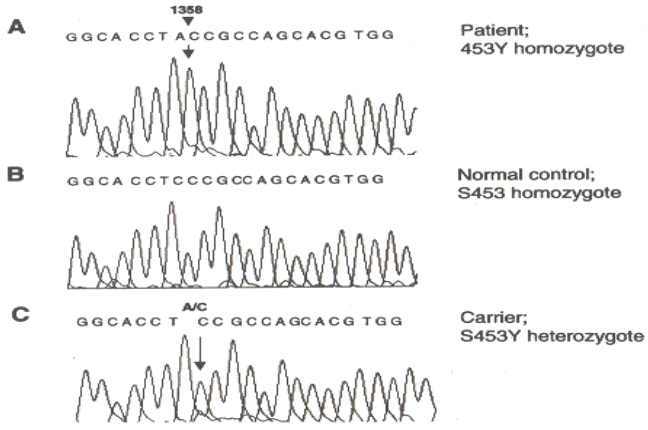

Fig. 4. Electropherograms showing the patient (A) homozygous for mutation 1358C→A (453Y), a normal control (B) homozygous for the 1358 C allele (S453) and a (C) heterozygous carrier, 1358C/A (S453Y), of the mutant allele. Nucleotide position 1358, from the first ATG of the alpha-mannosidase cDNA, is indicated by an arrowhead. Arrows indicate the positions of the 1358C→A mutation.

We present this case of alpha-mannosidosis because of its being a storage disease to remind that it should be considered in the differential diagnosis of MPS or other storage diseases with clinical findings like deafness, recurrent infections, hepatosplenomealy, facial coarsening and skeletal abnormalities. Further analysis of the disease by enzyme and DNA studies should be performed since accurate diagnosis is also important for genetic counseling and prenatal diagnosis.

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