Molecular Analysis in a Turkish Patient with Severe Form of Hurler Syndrome: Identification of a Novel c.826_828del3 Mutation

Hurler Sendromlu Türk Olguda Yeni Bir Mutasyon: C.826_828DEL3

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Abstract
Mucopolysaccharidosis type I (MPS I) is a lysosomal disease due to mutations in the gene encoding alpha-L-iduronidase (IDUA) leading to variable clinical phenotypes with progressive severe organomegaly, bone and neurological involvement in the most severe forms. A two-year-old Turkish patient born from consanguineous marriage had an enzymatic and urinary diagnostics suggested a MPS I phenotype. The genetic evaluation revealed c.826_828del3 mutation in the homozygous state, whereas her parents were heterozygous for this mutation. Because of the high frequency of consanguineous marriages in Turkey, identification of the novel mutations permits reliable genetic counseling of at-risk relatives and molecular prenatal diagnosis.

Keywords: **Mucopolysaccharidosis I; Mutation.**

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Özet
Mükopolisakkaridoz tip I (MPSI), alfa-1-iduronidaz geninde mutasyon sonucu gelişen; ilerleyici organomegali, kemik ve nörolojik tutuluma neden olabilen bir otozomal resesif geçişli, lizozomal depo hastalığıdır. İki yaşındaki kız olgumuz,kuzen anne ve babanın, yaşayan ikinci çocuğu idi. Hastanın kaba yüz görünümü ve idrarda artmış mükopolisakkarid atılımı MPSI, Hurler sendromunu düşündürdü. Lökosit içi alfa-1-iduronidaz aktivitesinin düşük olması ile hasta Hurler sendromu tanısını aldı. Moleküler çalışmalar, daha önce Hurler sendromu için literatürde tanımlanmamış olan c.826_828del3 mutasyonun varlığını gösterdi. Akraba evliliklerin sık olduğu ülkemizde, bu yeni mutasyon Hurler sendromunun prenatal tanısı açısından önemli olması nedeni ile bu olgu sunulmaya değer bulundu.

Anahtar Kelimeler: **Mutasyon; Mükopolisakkaridoz I.**

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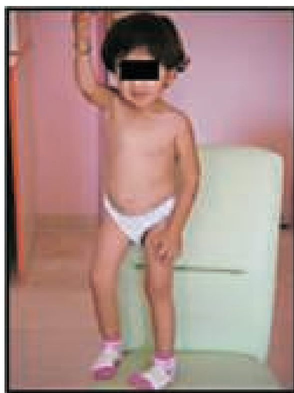
Introduction

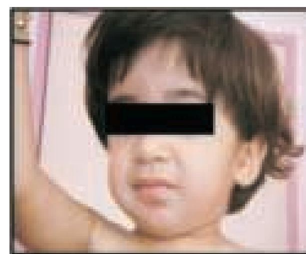
Mucopolysaccharidosis type I (MPS-I, OMIM# 252800) is an autosomal recessively inherited lysosomal storage disorder, resulting from a deficiency in the glycosidase, α-L-iduronidase (IDUA, E.C. 3.2.1.76). IDUA gene was cloned in 1990 and was localized to chromosome 4p16.3 (1,2). It is well known that α-L-Iduronidase is involved, at a specific step, in the degradation of the glycosaminoglycans (GAGs), dermatan, and heparan sulphate. Failure to degrade an iduronic acid residue from the non-reducing end of these glycosaminoglycans results in the intracellular accumulation of undegraded substrate within the lysosomes of affected cells and this is presumed to initiate the clinical manifestations of MPS I. It is believed that the underlying cause for the wide variation in MPS I patient clinical phenotype relates to the level of residual mutant α-L-iduronidase activity in patient cells, which in turn reflects a high level of molecular heterogeneity in IDUA gene mutations. Three MPS I patient clinical phenotypes have been reported, with different degrees of severity: Hurler syndrome (severe) Hurler–Scheie syndrome (intermediate severity) and Scheie syndrome (attenuated) (3, 4). These clinical phenotypes are now recognized as part of a spectrum of presentations, ranging from the archetypical severe form of the disorder to near normal presentation. Hurler syndrome patients typically display physical symptoms including coarse facial features, hepatosplenomegaly, joint stiffness, dysostosis multiplex, and short stature. In this severe form of the disorder onset is rapid and symptoms progressive, with patients suffering mental retardation and early death, often before 10 years of age.

Case Report

A two-year-old girl was clinically diagnosed as a severe form of Hurler syndrome. She was born normally to consanguineous parents. She has a healthy 15 years old brother and six years and one week old dead sister and brother.

At two years she weighed 10.95 kg (10 centile), measured 84.5cm height (25-50 centile) and head circumference was 50cm (90-97 centile). Her facial appearance was typical of patients with Hurler's syndrome, exhibiting a depressed nasal bridge, broad nasal tip, corneal clouding, and long upper lip with relative flattening of the philtrum (Pic 1). Her liver was palpable at 3 cm below the costal margin and radiography of the skeleton revealed dysostosis multiplex. Subsequently she developed an aortic valve insufficiency and vanishing of hearing. Her development was slightly delayed for age throughout (head control: 3-4 months, supported sitting: 6-8 months, unsupported sitting: 9-10 months, walking: 18-24 months). Her urine analysis for lysosomal storage disease revealed an increased excretion of mucopolysaccharidoses 20.4 mg/mol creatinine (N<16.4). Thereafter, a decreased alpha–iduronidase activity (2.87nmol/hour/mg protein (12-60)) was found.





Picture 1. Full body view of patient at the age of 2 years (left) and the facial appearance of the presented patient (right). Informed consent form was obtained from the patient's parents.

DNA extraction. Genomic DNA samples from the proband and her parents were extracted from peripheral lymphocytes using the Wizard Genomic DNA Purification kit (Promega, Madison, WI) according to the manufacturer instructions.

DNA amplification and sequence analysis. Mutation analysis of the IDUA gene (SeqRef Genebank: M95739, M95740) was carried out by amplification with a proofreading DNA Polymerase and direct sequencing of the whole coding and splicing site regions. Sequence

analysis was performed according to standard procedure (ABI Big Dye Terminator) followed by analysis on an ABI Prism 3100 Avant Automatic Sequencer (Applied Biosystems). Intronic primers were designed for both amplification and sequencing. Mutations detected by sequencing were further confirmed by a repeated PCR sequencing.

Molecular screening of IDUA gene of the proband revealed the occurrence of the deletion c.826_828del3 in the homozygous state, an in frame deletion of three nucleotides (GAG) codifying the aminoacid E276 (exon 7). This is a novel mutation not described in the literature yet (Figure 1-2). Two common polymorphisms were also identified in the homozygous state: p.A8 and p.A20 (exon 1). Molecular investigation of proband's parents confirmed their carrier status for the mutation c.826_828del3. This finding is consistent with consanguinity of parents and attests the high frequency of consanguineous marriages in Turkey.

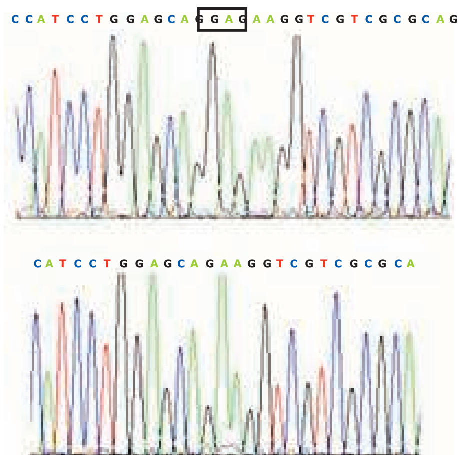


Figure 1. Results of sequence analysis for the region of exon 7 containin the deletion. Upper: wild type; bottom: proband c.826_828del3.

Figure 2. A multiple alignment of IDUA proteins of different species for the region containing the mutation identified is reported.

Discussion

Currently, over 100 disease-causing IDUA mutations have been described (5). Mutation frequencies vary worldwide, but W402X and Q70X are the two most frequent mutations found in European patients, being responsible for up to 70% of the alleles in some countries (6,7,8). The W402X and Q70X mutations have both been shown to produce no α-L-iduronidase protein and have therefore been described as "null alleles". This absence of α-L-iduronidase protein has been associated with a very severe clinical presentation.

To date, all patients with a nonsense mutation identified on both alleles have developed the severe form of MPS I. The phenotypes of patients with missense, insertion, deletion, or splice site mutations are much more variable. Missense mutations are the most likely to allow for some residual enzyme activity, and in particular, the R89Q mutation (9) has been identified in several mild patients even when in combination with a nonsense mutation. Conversely, most splice site and insertion/deletion mutations result in the severe phenotype unless in combination with a less severe missense mutation (10,11).

According to these general considerations we assumed that the novel deletion found in our patient is very likely a mutation causing disease. Indeed, c.826_828del3 is an in frame deletion of three nucleotides (GAG) that causes the loss of the Glutamic Acid at the position 276 of aminoacidic chain. The elevated conservation of the amino acid E276 in the evolutionary scale supports our hypothesis (Figure 2).

Moreover, bases deletion is generally associated to a severe MPS-I phenotype and the case of our patient supports this "premise". Although the large amount of novel and private mutations continuously identified in MPS-I patients make genotype-phenotype correlation a challenging effort, some insight into phenotypic expression may be obtained by observing the clinical severity of other patients with the same genotype (11, 12).

The complete screening of the whole coding and splicing site regions of IDUA gene has been demonstrated a good strategy, useful to identify novel mutations: the finding of the novel deletion c.826_828del3 in our patient have let us to plan a genetic counseling of at-risk relatives and to offer the possibility of a molecular prenatal diagnosis.

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