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Novel frameshift variant in the IDUA gene underlies Mucopolysaccharidoses type I in a consanguineous Yemeni pedigree

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ABSTRACT

Mucopolysaccharidosis type I (MPS I) is an autosomal recessive storage disorder that result as a consequence of a deficiency in the lysosomal hydrolase, a-L-iduronidase enzyme encoded by IDUA gene. Over a hundred causative variants in IDUA have been identified, which result in a progressive multi-systemic disease with a broad range of severity and disease progression reported across affected individuals. The aim of this study was the detection and interpretation of IDUA mutation in a family with two children affected with lethal MPS I. The IDUA gene was sequenced in the parents of two deceased children who had a clinical diagnosis of MPS I, to assess their carrier status and to help inform on risk in future children. The sequencing analysis was performed by PCR and bi-directional Sanger sequencing of the coding region and exon-intron splice junctions at Labor MVZ Westmecklenburg molecular diagnostics laboratory. A heterozygous c.657delA variant in exon 6 was identified in each parent, which is the most likely explanation for disease in their children. This report represents the first Yemeni family to have a molecular diagnosis for MPS I.

1. Introduction

Mucopolysaccharidosis type I (MPS I) is an autosomal recessively inherited lysosomal storage disorder, characterized by progressive multi-systemic disease. The clinical phenotype of MPS I has been grouped into three categories based on severity of the disease [1–3]: A severe form (Hurler syndrome) which involves mental retardation, skeletal deformities, stiff joints, hepatosplenomegaly, corneal clouding, and a shortened life expectancy [4]. An intermediate form (Hurler/Scheie syndrome) which involves skeletal deformities, severe organomegaly, usually limited bone involvement, and a variable life span with neurological involvement [5]. A mild form (Scheie syndrome), which is characterized by mild skeletal deformities, stiff joints, corneal clouding and a long life span without mental retardation [6–8].

MPS I is caused by variants that reduce or completely eliminate the lysosomal enzyme alpha-L-iduronidase (IDUA) enzyme [6,9,10] which is responsible for degradation of mucopolysaccharides. Deficiency of the IDUA enzyme results partial degradation and lysosomal accumulation of its substrates [1,11], which lead to progressive dysfunction of several organs [12,13]. Currently more than hundred mutations in the *IDUA* gene have been reported (Human Gene Mutation Database, http://www.hgmd.org/) [14,15]. High prevalence of common mutations p.W402X, p.Q70X and P533R has been confirmed [16–18]. Rare mutations including single base substitution, deletion, insertion, and splicing site mutation have been recognized [19], an indication of a high degree of allelic heterogeneity of *IDUA* mutation [18,20].

Identification of the specific mutations in affected patients and carriers is useful both for prenatal diagnosis, as well as genotype-phenotype correlations [21]. In this study we report a novel *IDUA* mutation in MPS I affected Yemeni consanguineous family.

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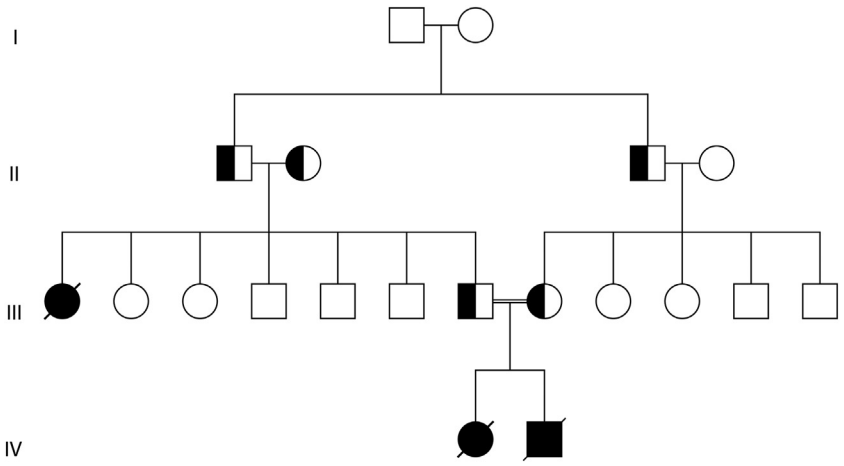

Fig. 1. Pedigree of MPS I family. Each generation is designated by roman numerals (I–IV). Squares and circles indicate male and female members, respectively. Half Shaded symbols indicate carrier individuals; full shaded symbols indicate affected individuals. Double lines indicate consanguineous mating.

2. Patients and methods

2.1. Patients

In this study a consanguineous Yemeni family with MPS I was investigated. The affected probands (Fig. 1 IV-1 and IV-2) underwent a clinical diagnosis prior to their death. Blood samples were collected from the two parents (Fig. 1 III-7 and III-8) after informed consent.

2.2. IDUA mutation analysis

Genomic DNA was isolated from peripheral blood using commercial kit (Qiagen miniprep, USA) according to the manufacturer's protocol. The extracted DNA from the two parents was submitted to the Labor MVZ Westmecklenburg molecular diagnostics laboratory in Germany for Sanger sequencing of the *IDUA* gene. All coding exons and only exon 6 of the *IDUA* gene for the father (Fig. 1 III-7) and the mother (Fig. 1 III-8) respectively, plus the exon-intron splice junctions were amplified by polymerase chain reaction (PCR). PCR fragments were sequenced using bidirectional Sanger sequencing.

Alamut Visual Version 2.6 software was used to analyze the variant against databases including dbSNP and EXAC.

3. Results

3.1. Clinical features and family history

A consanguineous first cousin Arabian Yemeni couple sought clinical attention and genetic counseling due to a family history of two deceased offspring with a clinical diagnosis of MPS I, as well as a sister with MPS I (Fig. 1). The couple (III-7 ad III-8) were not affected and did not have any other children. Clinical diagnosis of MPS I was made for the affected siblings (IV-1 and IV-2) based on the clinical features, family history and biochemical testing. Patients IV-1 and IV-2 showed classical hurler syndrome's signs and symptoms. They mainly suffered from cardiomegaly, coarse facial features, dysplasia, and intracranial hypertension. Both children died at ages 2 and 5 years respectively. Their paternal aunt (III-1) reportedly also suffered from similar clinical manifestations.

3.2. Molecular analysis

Molecular characterization of the IDUA gene by Sanger sequencing was done for individuals III-7 and III-8 (Fig. 1).

Sequencing analysis identified a heterozygous single base deletion in exon 6 of IDUA (c.657delA) in each parent, which is predicted to result in a frameshift of the protein sequence (p. Gly220Alafs*14). As the impact of this variant is deleterious to the function of the protein, this variant in the homozygous state is likely the cause of MPS in the offspring. However, DNA from the affected offspring was not available for testing to confirm this. The c.657delA mutation has not been reported in other MPS cases in the literature, but it was reported in 1/42210 European alleles in the ExAC database (http://exac.broadinstitute.org/variant/4-995534-AG-A). Other clinically irrelevant polymorphisms were detected (Table 1).

4. Discussion

MPS I is a rare genetic disorder with an estimated incidence of 1 case per 100,000 live births [12,13,22], it's characterized by a wide spectrum of disease with variable age of onset, progression, and organ involvement [23]. Without treatment, patients with the most severe phenotype will be exposed to a progressive deterioration of the musculoskeletal, cardiorespiratory, and central nervous system and, in most

Table 1
Polymorphisms characteristics and position in MPS I family.

Location	Position	Type	Nuc change	AA change	cDNA	Polyphen2	SIFT	Web reference
E1	187 (99)	C	T → G (homo)	H → Q [33]	c.99T > G	Benign	Neutral	rs10794537
E3	15 (314)	C	G → A (het)	R → Q (105)	c.314G > A	Benign	Neutral	rs3755955
E5	50 (543)	C	T → C (het)	N → N (181)	c.543T > C	N/A	N/A	rs6815946
I6	− 8	C	C → T (het)		c.590-8C > T	N/A	N/A	rs6848974
E8	109 (1081)	C	G → A (het)	A → T (361)	c.1081G > A	Benign	Neutral	rs6831280
E8	192 (1164)	C	G → C (het)	T → T (388)	c.1164G > C	N/A	N/A	rs6836258
I9	− 19	C	G → C (het)		c.1190-19G > C	N/A	N/A	rs150523349
E9	41 (1230)	C	C → G (het)	T → T (410)	c.1230C > G	N/A	N/A	rs11579097
E9	171 (1360)	C	G → A (het)	V → I (454)	c.1360G > A	Benign	Neutral	rs73066479
E10	65 (1467)	C	C → T (het)	R → R (489)	c.1467C > T	N/A	N/A	rs11592969

E: Exon, I: Intron, AA: amino acid.

Table 2
List of IDUA pathogenic variants causing MPS I in Middle Eastern populations.

No.	Population	Mutation	Reference
1	**Yemen**	**c.657delA**	**This study.**
2	Saudi Arabia	c.1525-1G > C	[27]
3	Saudi Arabia	p.Leu623Pro (c.1868T > C)	[27]
4	Saudi Arabia	p.Trp402 (c.1206G > A)	[27]
5	Saudi Arabia	c.1598_1599ins52	[27]
6	Kwait	p.Arg628* (c.1882C > T)	[27]
7	Tunisia	p.Y581X	[28]
8	Tunisia	p.F177S	[29]
9	Tunisia	p.P533R	[28]
10	Tunisia	p.L530fs	[29]
11	Tunisia	p.F602X	[28]
12	Tunisia	p.R628X	[28]
13	Tunisia	p.L578Q	[28]
14	Tunisia	c.1805delT	[30]
15	Turkey	p.Trp68* (c.203G > A)	[27]

The pathogenic variant of this study is made highlighted with bold text.

cases, die before the age of 10 years [22,24]. Patients with the mild phenotype have normal cognitive functioning and survive into adulthood; nevertheless 50% may be affected by cardiac valve abnormalities, joint contractures, corneal clouding, hernias, and hepatomegaly [8,24]. Treatments aimed at delivering functional IDUA enzyme to patients include bone marrow (BMT) or umbilical cord transplant and enzyme replacement therapy (ERT), which appear to be effective in slowing down the progression of the disease [25].

MPS I results from a mutation in *IDUA* gene and has an autosomal recessive mode of inheritance. A large number of pathogenic variants in the *IDUA* gene has been reported (see Table 2), including in Middle Eastern patients [26]. However to the best of our knowledge, this is the first study to sequence *IDUA* for mutations in Yemeni patients with MPS I. The identified disease causing c.657delA variant is not reported in any other patients with MPS I, but in seen in one allele in the ExAC database. The broad spectrum of variants in seen in Middle Eastern patients does not support a unique race-specific variant (a founder mutation) or variant spectrum, and highlights the need for comprehensive sequence analysis of the *IDUA* gene when MPS I is suspected in a Middle Eastern individual. Due to the rarity of the disease as well as the variability of clinical features, MPS I poses challenges for early clinical diagnosis. Molecular diagnosis via IDUA sequencing allows for early diagnosis and the potential to pursue available treatments which may slow down disease progression.

We performed in-silico analysis for the pathogenic variant, it is identified by SIFT as deleterious (http://sift.jcvi.org/) and probably damaging by Polyphen (http://genetics.bwh.harvard.edu/pph2/). The *IDUA* mRNA transcript of the mutated allele harbored an earlier premature translation termination codon, resulting in truncated *IDUA* protein at residue 229 of the total 653 amino acids (Fig. 2). A comparative in-silico protein modeling using SWISS-MODEL (http://swissmodel.expasy.org/) reveals the mutated IDUA and its' wild-type counterpart (Fig. 2). The virtual 3D modeling shows the magnitude of mutation impact on the simulated protein structure. The PTC would occur in exon 6 (of 14) and thus the mutant *IDUA* transcript is predicted not to reach the translation machinery. Rather it is predicted to be degraded by nonsense-mediated mRNA decay (NMD) (Fig. 2). NMD is a surveillance pathway that exists in all eukaryotes [31,32]. It targets mRNAs harboring PTCs for degradation [33], which if left intact, would lead to production of truncated proteins with predicted deleterious effects for the organism [34]. From a medical view, this suggests that the NMD pathway plays a significant role in modulating the phenotypic outcome of genetic disorders that are caused due to the presence of PTC [35,36].

In summary, this is the first study to sequence *IDUA* for pathogenic variants in a Yemeni family. We identified a novel pathogenic frameshift variant. This finding expands the pathogenic variant spectrum

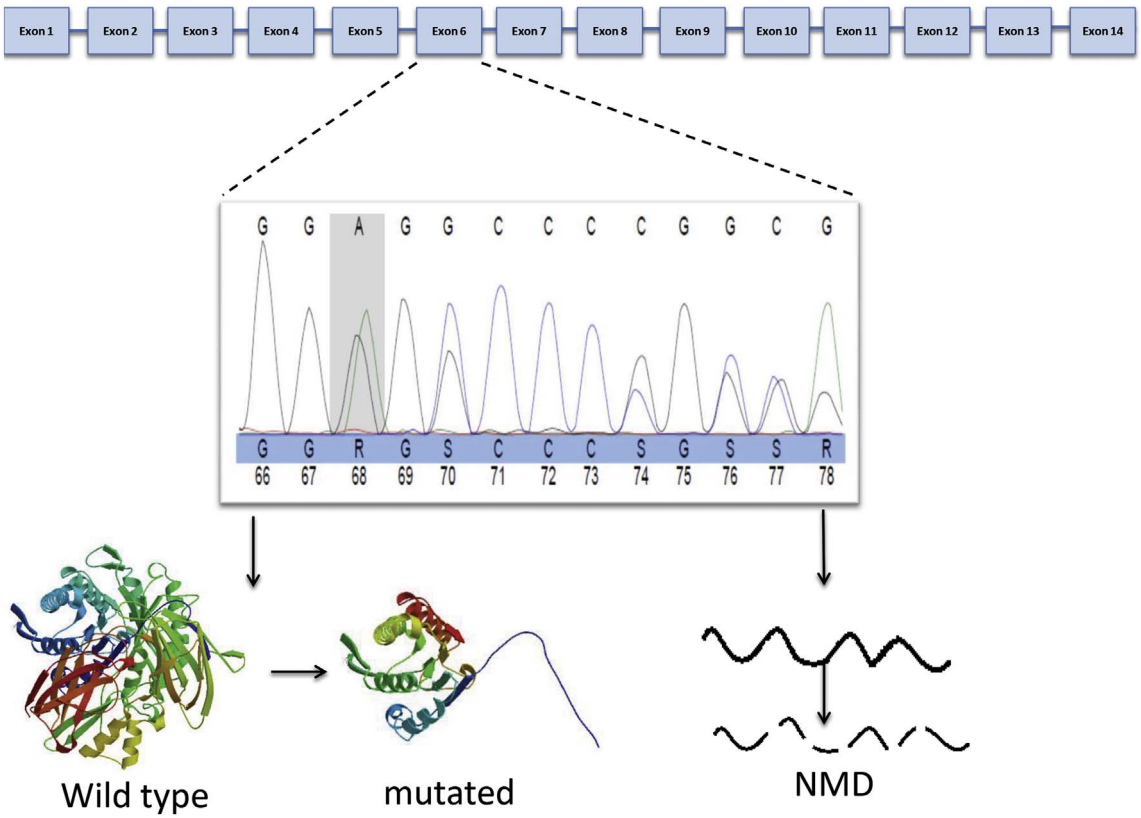

Fig. 2. Mutation analysis of the *IDUA* gene (c.657delA). Deletion of alanine leads to a premature stop codon, the affected allele will be either degraded by non-sense mediated mRNA decay (NMD) and in consequence the allele is functionless, or it can be translated causes the resulting protein to be truncated.

across Middle Eastern individuals and highlights the utility of early detection through molecular diagnostic for early intervention. Furthermore identification of this variant will facilitate accurate risk assessment as well as prenatal testing for future pregnancies.

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