Short Communication

Identification a novel mononucleotide deletion mutation in *GAA* in pompe disease patients

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Background: Mutations in the acid alpha-glucosidase (*GAA*) gene usually lead to reduced *GAA* activity. In this study, we analyzed the mutations of *GAA* and *GAA* enzyme activity from one sibling suspected Pompe disease and their first-degree relatives. **Materials and Methods:** In this cross-sectional study, *GAA* enzyme activity assay was assessed using tandem mass spectrometry. Polymerase chain reaction and Sanger sequencing were performed for *GAA* analysis. **Results:** *GAA* enzyme activity was significantly decreased in patients compared to the normal range ($P = 0.02$). Two individuals showed ten alterations in the *GAA* sequence, in which one of them (c. 1650del G) has not been previously described in the literature. A single Guanine deletion (del-G) was detected at codon 551 in exon 12. **Conclusion:** According to the literature, the detected change is a novel mutation. We hypothesized that the discovered deletion in the *GAA* might lead to a reduced activity of the gene product.

Key words: Acid alpha-glucosidase, novel mutation, polymerase chain reaction, Pompe disease

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INTRODUCTION

Pompe disease, a glycogen storage disease (GSD) Type II (OMIM 232300), is characterized by a deficiency of the normal function enzyme acid alpha-glucosidase (*GAA*) that results in intralysosomal accumulation of glycogen. Clinical heterogeneity is a pivotal characteristic of Pompe disease which reveals importance of investigations in different populations.[1,2] This heterogeneity occurs due to many mutations in the *GAA* gene which cause incomplete or complete lack of *GAA* activity and also different clinical manifestations.[2-4] The *GAA* is located on chromosome 17q25.2–25.3 which contains twenty exons and is highly polymorphic with a large number of neutral variations.[5] To the best of our knowledge, 351 disease-causing mutations have been described in the *GAA*, however, the leaky c.-32-13T>G (usually known as IVS1-13T>G) is the most frequent mutation among the Caucasian Pompe disease patients.[6,7] Therefore, in this study, we aimed to analyze the *GAA* and *GAA* enzyme activity from one sibling suspected Pompe disease and their first-degree relatives.

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MATERIALS AND METHODS

Study population

An Iranian couple with three children, a 26-year-old affected son, a 34-year-old affected daughter, and a 31-year-old healthy daughter were referred to our center, Isfahan, Iran. In this cross-sectional study, due to the very low prevalence of Pompe disease and also based on low sample size of previous studies, we investigated mutations of *GAA* gene in two patients with Pompe. Both patients are resident of Semnan Province. Research

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protocols and consent forms were approved by the Genome Research Center (Code: G-1263).

Enzyme activity and polymerase chain reaction

For confirmation of the diagnosis, an enzyme activity assay test to determine reduced or no activity of the *GAA* enzyme is required. *GAA* enzyme activity assay was assessed using tandem mass spectrometry.

Genomic DNA was extracted from total blood using DNA extraction Spin Kit (GE Healthcare UK Ltd., Buckinghamshire, UK) according to the manufacturer's instructions. Polymerase chain reaction and Sanger sequencing were performed for *GAA* analysis.

Statistical analysis

Data were collected and then SPSS for software (version 20.0; SPSS Chicago, IL, USA) was used for statistical analysis. The comparison of *GAA* enzyme activity between Pompe patients and the lower limit of normal range of *GAA* enzyme activity was performed using one-sample *t*-test.

RESULTS

Enzyme activity

Both patients had more than 17% reduction in the *GAA* activity (range: 0.13–0.19 nmol/spot*21 h) compared to the normal range (>0.9 nmol/spot*21 h) [Table 1]. The patients had elevated serum levels of creatine phosphokinase and liver enzymes (aspartate aminotransferase and alanine aminotransferase) ranging from 2 to 10-fold of the normal ranges.

Genotyping

Our study revealed ten alterations in the patients. According to Pompe Center, one of the alterations was novel, and nine of them were previously reported. These alterations in the *GAA* were eight single-nucleotide polymorphisms (SNP) and two mutations including one deletion mutation and one large deletion splice site mutation. The new alteration c.1650del G was found in two children of this family. Molecular analysis revealed two heterozygous mutations including a deletion of a single guanine in exon 12 at codon 551 and a large deletion in intron 1. Sequencing of *GAA* in other member of this family revealed that father, the healthy daughter and one affected of the children were heterozygous for the c. 32-13T>G mutation, while the mother was normal for this mutation. For this new deletion mutation, the both of father and healthy daughter were normal, but mother and affected children were heterozygous.

DISCUSSION

Our data confirmed the clinical manifestations of GSDII in the patients. In addition, the activity of *GAA* approved the molecular genetic results. The activity of *GAA* at PH 3.8 with and without specific inhibition was less than the normal ranges. Two patients had similar *GAA* activity and *GAA* alterations. Different clinical symptoms in these two patients may in part be clarified by the existence of other variants in *GAA* gene or other related genes. *GAA* mutations are located on different parts of the gene and include missense, nonsense, splicing, and both small and large deletions and insertions. Although most of the mutations related to GSDII are located on limited numbers of regions, some of them are common in especial ethnical populations.[8] The most common mutation is IVS1-13T>G, which seen in approximately 77% of patients with Pompe who are from different ethnic populations. The presence of this mutation result in improper splicing in 80%–90% of the *GAA* premessenger RNA splicing events.[9] Although this mutation was found in our cases, the father of this family was homozygous. In this regard, Musumeci *et al*.[10] reported six Pompe patients who were homozygous for c. 32-13T>G mutation. The new mutation c. 1650delG was heterozygous in two patients and their mother. The female patient showed higher severity in clinical symptoms compared to his brother. The low enzyme activity of *GAA* of patients was in line with the presence of the alteration in c. 32-13T>G and c.1650delG. However, further investigations in different populations will be required to establish these mutations in the *GAA* gene of Pompe patients.

CONCLUSION

According to the literature, the detected change is a novel mutation. We hypothesized that the discovered deletion in the *GAA* might lead to a reduced activity of the gene product.

Table 1: Measured acid alpha-glucosidase activity of the affected individual in comparison to the reference values, which shows significant differences

	Alpha-glucosidase measurement from dried blood specimen (nmol/spot*21 h)			Enzyme levels			Clinical manifestations	Spirometry	
	pH 3.8		pH 7.0	AST	ALT	CPK		FEV1	FEV1/FVC
	With acarbose	Without acarbose							
Patient 1	0.19	0.89	4.40	211	294	1925	Respiratory distress and muscle weakness	0.81	89.25
Patient 2	0.13	0.65	3.84	165	261	1212	Respiratory distress	0.83	90.25
Reference	0.9-7.2	1.5-10	1.8-17.1	0-45	5-40	25-200			

CPK = Creatine phosphokinase; AST = Aspartate transaminase; ALT = Alanine transaminase; FVC = Forced vital capacity; FEV1 = Forced expiratory volume in 1 s

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Conflicts of interest

There are no conflicts of interest.

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