Case Report

Detection of a Novel Mutation in the GAA Gene in an Iranian Child with Glycogen Storage Disease Type II

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Abstract

Glycogen storage disease II (GSDII or Pompe disease, OMIM # 232300) is an autosomal recessive hereditary lysosomal disorder. Mutations in the GAA gene usually lead to reduced acid α-glucosidase (acid maltase, GAA, OMIM *606800, EC 3.1.26.2) activity, which results in impaired degradation and subsequent accumulation of glycogen within lysosomes. We present an Iranian boy, who was diagnosed with GSDII based upon clinical and biochemical findings. A single adenine insertion (insA) was detected at codon 693 that leads to a predicted premature stop codon at codon 736 in the GAA gene. The parents were heterozygous for the same change. According to the human genome mutation database (www.hgmd.org) and lecture reviews, the detected change is a novel mutation. We suppose that the discovered insertion in the GAA gene might lead to a reduced activity of the gene product. This assumption is in agreement with biochemical and clinical signs in the patient.

Keywords: Acid α-glucosidase, glycogen storage disorder type II, Iranian, novel mutation, Pompe disease

Cite the article as: Galehdari H, Emami M, Mohammadian G, Khodadadi A, Azmoon S, Baradaran M. Detection of a Novel Mutation in the GAA Gene in an Iranian Child with Glycogen Storage Disease Type II . *Arch Iran Med.* 2013; **16(2):** 126 – 128.

Introduction

The Pompe disease, also has been termed to acid maltase deficiency (AMD) or glycogen storage disease type II (GSDII), is a rare (estimated in 1 of 40.000 births) inherited disorder of glycogen metabolism with autosomal recessive pattern resulting from defects in activity of the lysosomal hydrolase acid α-glucosidase (GAA, acid maltase) in all tissues of affected individuals.[1] The enzyme deficiency results in intra-lysosomal accumulation of glycogen of normal structure in numerous tissues.[2] The clinical presentation of GSDII encompasses of phenotypes, all of which include varying degrees of myopathy but differ with respect to age of onset, extent of organ involvement and rate of progression to death.[3]

To date, close to the 200 disease causing mutations have been described in the GAA gene, from them the leaky c.-32-13T>G (traditionally IVS 1-13T>G) is the most frequent mutation among the Caucasian GSDII patients.[4] But to our knowledge, there are no molecular reports from Middle East, especially from Iran.

Case Report

An Iranian couple with two children, a 10-month-old affected son and a 6-year-old healthy daughter, from southwest Iran referred to our gene test laboratory. The couple are third degree relatives and of Arab descent. Their affected son was diagnosed with glycogen storage disease II (GSDII) or Pompe disease. The primary diagnosis was established on the basis of clinical data such as massive cardiomegaly as seen on chest radiography, general muscle weakness, hypotonia, frequent pulmonary infections, and mild hepatomegaly.

A definite diagnosis usually requires an enzyme assay test to indicate reduced or no activity for the lysosomal enzyme acid α-glucosidase. GAA activity was tested from dried blood by measurements of α-glucosidase at pH 3.8 and pH 7.0, and with acrobase as an inhibitor. The enzyme activity of the patient confirmed classic Pompe disease (Table 1).

Blood from all family members was subjected for genome extraction, amplification, and subsequent sequencing of the entire exon and flanking intron regions. The primers were designed by Oligo7 software, according to the GAA gene sequence (NCBI: NT024871). All primers, size of the PCR products, and annealing temperatures for each primer pair are listed in Table 2. Generated PCR products were used for direct sequencing by Big Dyes Cycle Sequencing Kit and analyzed by an ABI automated sequencer 3770 (Applied Biosystems, USA).

Discussion

In order to identify the underlying molecular cause of GSDII, the entire 20 exons of the GAA gene were sequenced in this case of suspected GSDII. Molecular analysis revealed a homozygous insertion of a single adenine in exon 15 at codon 693 that encoded for the amino acid glutamine (CAG). Sequencing of the parent's GAA gene revealed that both were heterozygous for the mentioned mutation. Their healthy daughter showed no mutation (Figure 1). This finding confirmed the clinical manifestation of GSDII in the patient. In addition, the activity of α-glucosidase validated the molecular genetic results.

For individuals with late onset GSDII, prognosis depends on the age of onset. In general, the later the age of onset, the slower the progression of the disease.[5] Ultimately, prognosis is assessed by the extent of respiratory muscle involvement.[6]

To our knowledge, and according to the human mutation data-

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Accepted for publication: 16 May 2012

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

α-glucosidase measurement from dried blood specimen			
	pH 3.8		pH 7.0
Patient	With acrobase (nmol/spot*21 hours)	Without acrobase (nmol/spot*21 hours)	nmol/spot*21 hours
	0.09	0.68	5.90
Reference	> 0.9	>1.44	> 1.8

Table 2. Sequences of the forward and reverse primers used for separate amplification of exons in the GAA gene.

Exon	Forward primer	Reverse primer	Size (bp)	Annealing temperature (°C)
1	5-TCTCTGACCCCAGAGGAACCGGCA-3	5-CTAGGCACGTTCAAACCCGCT-3	653	64.0
2	5-ACAGCAGGGCAACACCCACCCT-3	5-ACATGCACCCCACCCTTGTGAGG-3	817	62.0
3	5-GTCCCACATCCATGTGTGGCTGCA-3	5-CTGCCGTGTGAGAAATGCGCGTCG-3	320	62.0
4	5-TGTCCCAGGGTCAGTGTGCTG-3	5-GGCATTGCTGTTTAGCAGGAACAC-3	478	62.0
5	5-GAGCACCTCAGTCCCCTGATGC-3	5-CCTTAGGAGCACAGAAGGCCA-3	421	62.0
6	5-TGAGTCAGGCTTAGCACGGCTT-3	5-TATGGCGGCATGAACGGGTATCCT-3	379	59.0
7	5-CTGCCCTTAGCTGGAGGTCGA-3	5-CGGAAGCCATCCTTGTTGAACGTG-3	457	59.0
8	5-CTATCACCCGCCAGGTGGTGGA-3	5-AAGACCCTCAGAAAACATCCTCG-3	391	61.0
9	5--AGGCGTGTGCAGGCATGTGCA-3	5-GGGCCATTCCTTGTAGGAGCCACC-3	439	62.0
10	5-AGAGCTGCTCATTGACCTCCAG-3	5-ACGTAGGGTGGGTTCTCCAGCTC-3	456	63.0
11	5- TGGTGGCTGAGTTCCATGACCAGG-3	5- GCACTGCTTGGCTGAGTCTCCCA-3	406	63.0
12	5- CAGGTTCCCGGGTAACGCCA-3	5- CAGCTTCAGGCTGGGGTGCA-3	353	63.0
13	5-CAACTGTGCCCGCAGACATGGGCA-3	5-AACTGCAGGATTTCTGGGAGG-3	399	60.0
14	5-CCACTTAGCAGGTGGGGCTCTG-3	5-GGAGAGCTGCACTTCTCAGCCACC-3	359	61.0
15	5-AATCCCACCCCTGCTGGAGAAGCA-3	5-ACTCAGCGGCAACCTGCTGGGT-3	398	61.0
16	5- TGCCCGTCTGACCTGAGTCCTCCA-3	5- AGGCTGATGGGTGCGGAGCA-3	425	61.0
17	5-AGGGTCCCTACCTACAGTGAGC-3	5-GGCTCCTTGATAACCTACACTGC-3	463	59.0
18	5-TGGGGAAGGTCTTGGGTCATCAC-3	5-GTGTACACGAAAGGGCAGAGTGC-3	515	59.0
19	5-TGGGGTCCTAGAGTGAGCAGTGG-3	5-ACAGCCTGTGTGGCCTGACC-3	486	59.0
20	5′-AACCGGGTGCGAAGCATCCCA-3	5′-AAGGAGCTGCCTCTGTTCCAGG-3	843	59.0

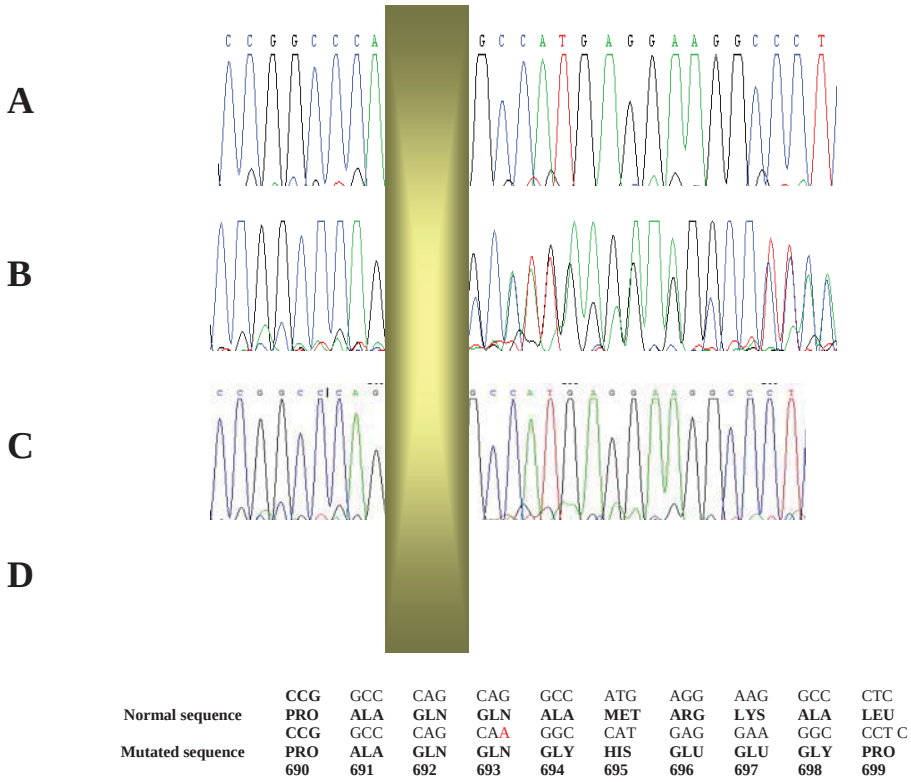

Figure 1. Autoradiogram of the sequencing results from: **A)** the healthy child, showing a normal sequence; **B)** the father of the affected boy, who was heterozygote for the insertion of a single adenine; **C)** the affected child, with homozygous insertion at codon 693. **D)** Partial reference sequence of the GAA gene. The position of the inserted adenine at codon 693 (CAG) is marked in red.

base for the GAA gene, the described nucleotide insertion represents an unreported mutation that leads to a predicted premature stop codon at codon 736 (http://www.hgmd.org), which can be definitely estimated by functional analysis.

To date, approximately 200 mutations have been described in the human GAA gene.[6] A number of these nucleotide changes are pathogenic, whereas others are marked as polymorphisms.[1–7] GAA gene mutations are located throughout the gene and include missense, nonsense, splicing, and both small and large deletions and insertions. It seems that the majority of mutations which cause GSDII are regional, whereas some are common in certain ethnic groups.[8] The most frequent mutation is IVS1-13T>G, which occurs in approximately 77% of patients with adult-onset GSDII who are from diverse ethnic backgrounds. A genotype-phenotype correlation has been identified.[1–9]

In the present study, we describe for the first time the molecular report of an Iranian boy with GSDII. This finding will expand the knowledge about pathogenic mutations in the GAA gene and might be useful for molecular screening of the disease in Iran. However, large size sample studies with Iranian GSDII patients are needed to determine the frequency and distribution of mutations in the GAA gene in Iran.

References to electronic databases

Pompe disease (glycogen storage disease II): OMIM #232300. Acid α-glucosidase: OMIM*606800; EC 3.1.26.2. OMIM Gene map locus for Pompe: http://www.ncbi.nlm.nih.gov/Omim/getmap.cgi?l232300. Pompe disease mutation database: http://www.hgmd.org

Acknowledgment

We thank Mr. Baha Salehi for his kind technical assistance.

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