ORIGINAL ARTICLE

Molecular features of 23 patients with glycogen storage disease type III in Turkey: a novel mutation p.R1147G associated with isolated glucosidase deficiency, along with 9 *AGL* mutations

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Glycogen storage disease type III (GSD III) is an autosomal recessive disorder caused by deficiency in the glycogen debranching enzyme (gene symbol: *AGL*) with two enzyme activities: transferase and glucosidase. A missense mutation causing isolated glucosidase deficiency has never been reported. In this study, we examined 23 patients of Turkish ancestry and identified a novel missense mutation p.R1147G with isolated glucosidase deficiency, along with nine *AGL* mutations: six nonsense mutations (p.W373X, p.R595X, p.Q667X, p.Q1205X, p.W1327X and p.Q1376X), one deletion (c.1019delA) and two splicing mutation (c.293+2T>G and c.958+1G>A). As p.R1147G impaired glucosidase activity, but maintained transferase activity *in vitro*, a 12-year-old girl homozygous for p.R1147G was diagnosed with having isolated glucosidase deficiency. Of nine other mutations, p.W1327X and c.1019delA were recurrent, whereas seven mutations were novel. Six patients with p.W1327X were all from two nearby cities on the East Black Sea and shared the same *AGL* haplotype, indicating a founder effect in Turkish patients. Patients with the same mutations had identical haplotypes. Our results provide the first comprehensive overview of clinical and molecular features of Turkish GSD III patients and the first description of the missense mutation associated with isolated glucosidase deficiency.

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INTRODUCTION

Glycogen storage disease type III (GSD III; MIM #232400) is an autosomal recessive inherited disorder characterized by fasting hypoglycemia, growth retardation, hepatomegaly, progressive myopathy and cardiomyopathy.^{1,2} GSD III is caused by a deficiency in the glycogen debranching enzyme, which has two independent catalytic activities: oligo-1, 4-1, 4-glucantransferase (EC 2.4.1.25) (transferase) and amylo-1, 6-glucosidase (EC 3.2.1.33) (glucosidase). Both activities are absent in the liver and the muscles in most patients with GSD III, which is named as subtype IIIa. Approximately 15% of the patients lose both two enzyme activity solely in the liver (subtype IIIb). Very few patients have been categorized to have selective loss of one of the two enzyme activities: isolated transferase deficiency (subtype IIId) and isolated glucosidase deficiency. Subtype IIIf was originally described as a reverse type of IIIb; one patient lost enzyme activity in muscle only.^{3,4} However, such patients have never been reported after the original patient, and the original meaning of IIIf apparently fell into oblivion. On the other hand, isolated glucosidase deficiency used to

be labeled as other⁴ or IIIf,⁵ but at present, it has been named as IIIf.⁶ The molecular analysis of patients with isolated glucosidase deficiency has never reported. In this study, we present clinical and genetic features of a patient with isolated glucosidase deficiency. *In vitro* functional analysis of p.R1147G, which was identified in patient 1, has been reported elsewhere.⁷

A gene coding human glycogen debranching enzyme (gene symbol: *AGL*) has been shown to be 85 kb in length and composed of 35 exons, encoding a 7.0-kb mRNA.⁸ Molecular analyses of GSD IIIa and IIIb have been performed in several ethnic populations and over 60 different *AGL* mutations have been reported to date (Human Gene Mutation Database; <http://www.hgmd.cf.ac.uk/ac/index.php>). Whereas only two cases have been reported in IIId,^{9,10} none have been reported in isolated glucosidase deficiency.

Depending on ethnic groups, the spectrum of *AGL* mutations in GSD III varies. In ethnic groups with high rate of consanguinity, prevalent mutations have been reported. For example, specific mutations are prevalent in North African Jewish population and in an

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Table 1 Clinical and genetic features of 23 Turkish GSD III patients

Patient number	Age	Gender	Consanguinity	Birth place	Hepatomegaly	Liver biopsy	Cardiomyopathy	Clinical muscle involvement	Plasma CK (IU)	Weight for height (%)	Height Z-score	Hypoglycemia	Other features	Enzyme activity	AGL Subtype	mutations
1	14y 5m	F	+	East Black Sea (ARTVIN)	+	Glycogen+fibrosis	–	–	79	100	–0.66	–	Tuberculosis infection	0	IIlc	p.R1147G/p.R1147G
2	11y	M	+	Middle Black Sea (SINOP)	+	Glycogen	+	+	797	129	–3.3	+	PSMR, frequent infection, cryptorchidism	0	IIla	p.W373X/p.W373X
3	3y	M	+	East Anatolia (VAN)	+	Glycogen+fibrosis	–	–	523	100	–1.09	–		0	IIla	p.R595X/p.R595X
4	10y	M	+	Unknown	+	Not done	–	–	NA	NA	NA	+		0	IIla	p.Q667X/p.Q667X
5	6y	M	+	Unknown	+	Not done	–	–	NA	NA	NA	+		0	IIla	p.Q667X/p.Q667X
6	16y 7m	F	+	East Anatolia (KARS)	+	Glycogen+fibrosis	+	+	3292	90	–2.78	+		0	IIla	p.Q1205X/p.Q1205X
7	7y 1m	M	+	South East Anatolia (DIYARBAKIR)	+	Glycogen	–	–	3066	101	1.01	+	PSMR	0	IIla	p.Q1376X/p.Q1376X
8	2y 4m	F	+	South East Anatolia (DIYARBAKIR)	+	Not done	NA	–	113	104	–0.42	–	Sibling of patient 7	0	IIla	p.Q1376X/p.Q1376X
9	20y	F	+	South Anatolia (ADANA)	+	Not done	NA	–	NA	NA	NA	+		0	IIla	p.Q1376X/p.Q1376X
10	4y 2m	M	+	Middle Anatolia (SIVAS)	+	Not done	–	–	934	123	–1.76	+		0	IIla	c.293+2T>G/c.293+2T>G
11	5y 6m	M	+	East Anatolia (ERZINCAN)	+	Glycogen	+	–	803	102	–0.69	–	Frequent enteritis	0	IIla	c.958+1G>A/c.958+1G>A
12	55 y	F	+	East Black Sea (GİRESUN)	+	Glycogen	+	+	1900	95	–0.38	–	Diabetes mellitus, macroglossia	0	IIla	p.W1327X/p.W1327X
13	19y 5m	M	+	East Black Sea (ORDU)	+	Glycogen	+	–	2783	127	–1.3	–	Cryptorchidism	0	IIla	p.W1327X/p.W1327X
14	15y 7m	M	–	East Black Sea (ORDU)	+	Glycogen+fibrosis	+	–	2049	110	0.64	+		0	IIla	p.W1327X/p.W1327X
15	27y	F	+	East Black Sea (ORDU)	+	Glycogen+fibrosis	+	–	3090	158	–0.35	+		0	IIla	p.W1327X/p.W1327X
16	19y	F	+	East Black Sea (ORDU)	+	Not done	+	+	566	86	0.05	+	PSMR, deafness, polydactyly, died at 19 y	0	IIla	p.W1327X/p.W1327X
17	15y 7m	M	+	East Black Sea (ORDU)	+	Not done	+	+	1005	85	–1.01	–	Nephromegaly, sibling of 16 y	0	IIla	p.W1327X/p.W1327X
18	11y	F	+	East Black Sea (TRABZON)	+	Not done	–	+	1304	100	–2.8	+	Frequent infection	0	IIla	c.1019delA/c.1019delA

Table 1 Continued

Patient number	Age	Gender	Consanguinity	Birth place	Hepatomegaly	Liver biopsy	Cardiomyopathy	Clinical muscle involvement	Plasma CK (IU)	Weight for height (%)	Height Z-score	Hypoglycemia	Other features	Enzyme activity	AGL subtype	AGL mutations
19	3y	F	+	Unknown	+	Not done	—	—	NA	NA	NA	+	—	0	IIla	c.1019delA
20	2y	F	—	Mother: East Black Sea (TRABZON) father: Middle Anatolia (KONYA)	+	Glycogen+fibrosis	—	—	358	111	0.1	—	—	0	IIla	c.1019delA
21	19y	F	+	Middle Anatolia (SIVAS)	—	Glycogen+fibrosis	—	+	1039	106	−0.36	—	PSMR	0	IIla	c.1019delA
22	2y	M	—	Unknown	+	Not done	—	—	NA	NA	NA	+	Dead	0	ND	c.1019delA
23	1y	M	+	Unknown	+	Not done	—	—	NA	NA	NA	+	—	0	ND	c.1019delA

Abbreviations: CK, creatine phosphokinase; F, female; GSD, Glycogen storage disease; M, male; m, month; NA, not available; ND, not determined; PSMR, psychomotor social retardation; y, year. Parents of patient 14 came from the same village. Patients 4, 5, 19, 22 and 23 were living in Germany.

isolate such as Faroe Islands.^{11,12} On the contrary, genetic heterogeneity has been shown in other ethnic groups, such as Japanese,¹³ Italian¹⁴ and Caucasian.¹⁵ In Turkey, the incidence of consanguineous marriages is generally high. However, we reported three different *AGL* mutations in three patients in Turkey: two splicing mutations c.846+5G>A (IVS7+5G>A) and c.2681+3dup (IVS21+5insA),¹⁶ along with one deletion (c.2474delC).¹⁷ To investigate further molecular characteristic in Turkey, we examined 23 new cases with Turkish GSD III.

MATERIALS AND METHODS

Patients

A total of 23 Turkish GSD III patients from 21 unrelated families were investigated. Patients 7 and 8 were siblings. Patients 16 and 17 were siblings as well. Clinical features were shown in Table 1. A total of 18 patients lived in Turkey and 5 in Germany. The patients were confirmed as having deficient debranching enzyme activity in peripheral blood cells by the method of Shin.¹⁸ Consanguinity was ascertained in 18 out of 21 families. The methods to evaluate cardiomyopathy were electrocardiography and echocardiography. The study was approved by local ethics committees and performed with the patients' and their families' informed consent.

Patient 1 is one of three children in a consanguineous family. Her parents were first cousins. Her sister died at birth and her brother died at age of 7 months due to cardiac insufficiency. She was first noticed to have hepatomegaly at age of 1 year and 3 months. Liver biopsy was performed at age 2 years and histological examinations showed glycogen accumulation and fibrosis. She suffered from tuberculosis when she was 9 years and 6 months old, and was treated for 6 months. Now patient 1 is 14 years and 5 months old, her physical and mental development have been normal. Her present clinical symptom is mild hepatomegaly only. She has experienced no episodes of hypoglycemia and has not showed muscle weakness on physical examination. Her electrocardiogram and echocardiogram are normal. Her plasma creatine phosphokinase level is 79 U l^{−1} (normal range: 62–287). Aspartate aminotransferase is 58 U l^{−1} (normal range: 5–32), alanine aminotransferase 84 U l^{−1} (normal range: 7–35), uric acid 3.7 mg per 100 ml (normal range: 2.6–6.0), triglycerides 218 mg per 100 ml (normal range <150), cholesterol 260 mg per 100 ml (normal range: <220) and lactate 0.8 mmol l^{−1} (normal range <2).

DNA sequence analysis of *AGL*

Genomic DNA was isolated from peripheral blood leukocytes. The full coding exons, their relevant exon–intron boundaries and the 5′- and 3′-flanking regions of the patients' *AGL* were sequenced directly, as described previously.¹³ The nucleotides of *AGL* cDNA were numbered according to *AGL* isoform 1 (GenBank accession no. NM_000642).

Point mutations identified in patients were verified using restriction fragment length polymorphism (RFLP). RFLP analyses to detect p.W1327X and c.1019delA were described previously.^{16,19} Besides, a pair of primers (Table 2) were used for PCR and each specific restriction endonuclease was added to digest PCR products. Restriction digests were analyzed on a polyacrylamide gel. A total of 50 Turkish control subjects were examined by RFLP in the same manner to rule out the possibility that they are mere polymorphisms in controls.

Haplotype determination in *AGL*

A total of 25 single nucleotide polymorphisms (SNPs) in *AGL* were determined, as described previously.¹⁶ SNPs numbers were cited according to database of Single Nucleotide Polymorphisms (dbSNP Build 130) available from <http://www.ncbi.nlm.nih.gov/SNP/>.

Bioinformatical analysis

Alignment of amino acid sequences from various species was performed using ClustalW2 (<http://www.ebi.ac.uk/Tools/clustalw2/>).²⁰

RESULTS

We identified 37 mutant alleles (Table 1) and found 10 different *AGL* mutations (Table 3) in Turkish patients. Eight mutations were novel

Table 2 RFLP detection for *AGL* mutations

Location	Mutation	PCR primer (5'→3')	Restriction enzyme	Fragment size (bp)
Exon 27	c.3439 A>G p.R1147G	F: TTTAGCATTTGCGGGTACCC R: CAGGCAAAGGAGCAGAATC	<i>MspI</i>	Normal: 206 Mutant: 140+66
Exon 10	c.1118 G>A p.W373X	F: AGCAATTGAAGATGCTGTA <u>ACT</u> R: gatgcaaggaaaagcagcag	<i>SpeI</i>	Normal: 129 Mutant: 108+21
Exon 15	c.1783 C>T p.R595X	F: ctcttctggacataaataatg R: TCCAACAGGTTCTCCTCCATCTC	<i>DdeI</i>	Normal: 126 Mutant: 102+24
Exon 16	c.1999 C>T p.Q667X	F: AGTACAAGAGGCTATGATGAATTAGTGCCTGAT R: gcatggatacatcttaattacaatg	<i>BclI</i>	Normal: 100+30 Mutant: 130
Exon 28	c.3613 C>T p.Q1205X	F: acagGATCAGCCATTGTTGAAGTTGTA R: caattgcaaggtttaatttcacc	<i>BsrGI</i>	Normal: 154+25 Mutant: 179
Exon 31	c.4126 C>T p.Q1376X	F: AAG GCT ATA AAG GTC TCA TAT G R: ATTGCTATGGTAAATAGGCCTC AGCT	<i>PvuII</i>	Normal: 177+26 Mutant: 203
IVS4	c.293+2T>G	F: AACTTAATCTGCAACAATCTGG R: tacagtaacatttacaagttagg	<i>BslI</i>	Normal: 131 Mutant: 84+47
IVS8	c.958+1G>A	F: GGGAGGATATTTTCCAAAGC R: aggaacattctagtatgtatccgata	<i>EcoRV</i>	Normal: 120 Mutant: 95+25

Abbreviations: F, forward; R, reverse; RFLP, restriction fragment length polymorphism.
Exon sequences are represented by uppercase letters and introns by lowercase letters.
Mismatch nucleotides are underlined.

Table 3 *AGL* mutations and haplotypes constructed by 25 SNPs

Polymorphism	SNP number	p.R1147G	p.W373X	p.R595X	p.Q667X	p.Q1205X	p.Q1376X	c.293+2T>G	c.958+1G>A	p.W1327X	c.1019delA
–10 in exon3	rs2307130	g	a	a	a	g	a	g	a	a	g
IVS3+85	rs663848	t	t	t	t	t	c	t	t	c	c
–2 in Ex4'	rs2307129	c	c	t	t	c	c	c	t	c	c
IVS4–122	rs3766593	t	t	t	t	t	t	t	t	t	t
IVS5–53	rs535461	c	c	c	c	c	g	c	c	c	c
IVS6–73	rs903126	g	g	a	a	g	a	g	a	a	a
p.L298L	rs2230306	t	t	t	t	t	c	t	t	c	ND
IVS8–18	rs634880	a	a	a	a	a	g	a	a	g	g
p.R387Q	rs17121464	R	R	R	R	R	R	R	R	R	R
IVS12+74	rs3736295	t	t	c	c	t	c	c	c	c	c
IVS12–125	rs2291637	a	a	t	t	a	t	t	t	t	t
IVS12–44	rs2291638	ND	g	a	ND	ND	a	ND	ND	a	a
IVS13–70	rs2392077	g	g	c	c	g	c	c	c	c	c
IVS16+8	rs3736296	c	c	t	t	c	t	t	t	t	t
IVS18+215	rs1389381	c	ND	c	a	c	c	c	c	c	c
IVS21+124	rs2234707	a	a	g	g	g	g	g	g	g	g
IVS22+11	rs555929	a	a	a	a	a	g	g	a	g	g
IVS23–121	rs2035960	g	g	a	a	g	a	a	a	a	a
IVS23–21	rs2035961	a	a	t	t	a	t	t	t	t	t
p.P1067S	rs3753494	P	P	P	P	P	P	P	P	P	P
p.G1115R	rs2230307	G	G	G	G	G	G	G	G	G	G
p.R1253H	rs12043139	R	R	R	R	R	R	R	R	R	R
IVS29+45	rs2274570	g	g	g	g	a	g	g	g	g	g
IVS29+53	rs1541041	t	t	t	t	a	t	t	t	t	t
IVS32–97	rs2296885	g	g	g	g	a	g	g	g	g	g

Abbreviations: ND, not determined; SNP, single nucleotide polymorphisms.

and two recurrent. All 10 mutations were verified by RFLP analyses and were not found in 50 normal Turkish controls (100 chromosomes).

Patient 1 was homozygous for a missense mutation in exon 27. Sequencing analysis showed an A-to-G substitution at nucleotide 3439, which replaces arginine by glycine at codon 1147 (p.R1147G).

RFLP analysis with *MspI* showed that patient 1 was homozygous for p.R1147G. No mutations except p.R1147G have been found in patient 1. Moreover, comparison of amino acid sequences among various species showed that arginine at codon 1147 was well preserved during evolution (Figure 1).

Human	GIYARYNCRDAVW
Chimpanzee	-----
Cow	-TH-----
Horse	-TH-----
Rabbit	-T-----
Dog	-----
Mouse	-----
Rat	-----
Chicken	-T-----
Fruit fly	-T-----
Mosquito	-QN-F-----
Zebra fish	-VG-F-----
C.elegans	-G-----T-
Yeast	-RNP--A---A-

Figure 1 Comparison of amino acid sequences around human 1147 amino acid of glycogen debranching enzymes from various species. Identical amino acids are represented as -. The human arginine at codon 1147 and corresponding amino acids are enclosed within a box. The species and the NCBI accession numbers are as follows: Human (*Homo sapiens*), NP_000019; Chimpanzee (*Pan troglodytes*), XP_524777; Cow (*Bos taurus*) predicted protein, XP_595566; Horse (*Equus caballus*), NP_001103778; Rabbit (*Oryctolagus cuniculus*), NP_001075716; Dog (*Canis familiaris*), XP_537057; Mouse (*Mus musculus*), NP_001074795; Rat (*Rattus norvegicus*), NP_001102034; Chicken (*Gallus gallus*) predicted protein, XP_422317; Fruitfly (*Drosophila melanogaster*), NP_726062; Mosquito (*Anopheles gambiae*), XP_321957; Zebra fish (*Danio rerio*) predicted protein, XP_696194; *Caenorhabditis elegans* hypothetical protein, NP_496984; Yeast (*Saccharomyces cerevisiae*), NP_015510.

Sequence analysis of patient 2 showed a G-to-A substitution at nucleotide 1118 in exon 10, resulting in premature termination at codon 373 (p.W373X). Patient 3 was a homozygote for p.R595X. In sequencing, a C-to-T substitution at nucleotide 1783 was found, which changes arginine to stop codon at codon 595 (p.R595X). Sequence analysis of patients 4 and 5 showed a C-to-T substitution at nucleotide 1999 in exon 16, causing premature termination at codon 667 (p.Q667X). Patient 6 had a C-to-T substitution at nucleotide 3613 in exon 28, replacing glutamine by a stop codon (p.Q1205X). Patient 7 was shown to be homozygous for a nonsense mutation. Sequencing analysis showed a C-to-T transition at nucleotide 4126 in exon 31, resulting in substitution of glutamine at codon 1376 by termination codon (p.Q1376X). RFLP analysis with *PvuII* indicated that patients 7, 8 and 9 were homozygotes for p.Q1376X. Patient 10 was homozygous for a T-to-G substitution at the second nucleotide of intron 4 (c.293+2T>G). Sequence analysis of patient 11 indicated a G-to-A substitution at the first nucleotide of intron 8 (c.958+1G>A).

Sequence analysis of patient 12 revealed a G-to-A substitution at nucleotide 3980 in exon 31 that replaces tryptophan by termination codon at codon 1327 (p.W1327X). RFLP analysis with *XbaI*, as described previously,¹⁹ indicated that six patients 12, 13, 14, 15, 16 and 17 were homozygous for p.W1327X.

In patient 18, sequencing analysis revealed deletion of A at nucleotide 1019 in exon 9 (c.1019delA), leading to a premature termination because of frameshift. RFLP analysis with *EcoRI*, as described previously,¹⁶ showed that patients 18 and 19 were homozygotes and patient 20 was heterozygote for c.1019delA. Sequencing analysis did not detect the other mutation of patient 20.

No mutations were detected in patients 21, 22 and 23. Patients had neither c.846+5G>A, c.2681+3dup nor c.2474delC, each of which was reported in Turkish GSD III. In addition, mutations in exon 3, reportedly associated with GSD IIIB, were not detected in any of 23 patients.

Figure 2 Geographic distribution of glycogen storage disease type III (GSD III) patients in Turkey. Figures in circles indicate patient number. Patients 4, 5, 19, 22 and 23 are not shown, because they live in Germany.

Geographic distribution of patients in Turkey was shown in Figure 2. Six patients with p.W1327X were all from two nearby cities on the East Black Sea.

AGL haplotype analysis of 10 mutations was shown in Table 3. Six patients with p.W1327X shared the same haplotype. Patients with same mutations, that is, two with p.Q667X, three with p.Q1376X and three with c.1019delA had identical haplotypes. These results indicated that patients with same mutations were descendants from a common ancestor in Turkey. Moreover, each haplotype had at least one different SNP, although haplotypes with p.R595X and c.958+1G>A were identical except rs2291638, which we could not determine in patient 11 with c.958+1G>A.

DISCUSSION

We have shown the allelic heterogeneity of AGL mutations in patients of Turkish ancestry. Our genetic analysis of 23 Turkish patients revealed 10 different mutations. Five novel nonsense mutations (p.W373X, p.R595X, p.Q667X, p.Q1205X and p.Q1376X,) are predicted to lead to premature termination, which completely abolishes the enzyme activity. Those premature stop codons are likely to be recognized by nonsense-mediated mRNA decay machinery, leading to absence of AGL mRNA. Two splicing mutations (c.293+2T>G and c.958+1G>A) are predicted to impair normal splicing, because the 5'-dinucleotides in the donor splice site should be GT for normal splicing. At the second nucleotide of the donor splice site in intron 4, the substitution by a different nucleotide (c.293+2T>A) was reported to cause aberrant splicing.²¹ Computational splice site analysis indicated that both mutations completely abolished the function of the donor splice site, suggesting that the mutation is likely to obliterate the splicing.²²

A novel AGL missense mutation p.R1147G was identified in patient 1. As far as arginine at codon 1147 is concerned, this amino acid is conserved across species and located in the C-terminal half of AGL. In addition, site-directed mutagenesis study in yeast glycogen debranching enzyme showed that amino acid substitutions in the C-terminal half lost glucosidase activity, but retained transferase activity.²³ Moreover, we expressed p.R1147G mutant and normal AGL proteins in COS cells transiently, and measured both glucosidase and transferase activities.⁷ The p.R1147G exhibited negligible glucosidase activity, but retained 40% of transferase activity compared with wild AGL. p.R1147G might alter the tertiary conformation of the enzyme and slightly reduce the transferase activity. However, transferase activity level is not deficient and this is different from IIIA.

Interestingly, p.R1147G displayed increase ubiquitination, though protein stability was not altered. We conclude that the diagnosis of patient 1 is isolated glucosidase deficiency. In the literature, there have been only one twin case with isolated glucosidase deficiency.⁴ To our knowledge, p.R1147G is the first missense mutation associated with isolated glucosidase deficiency. Clinically, patient 1 had mild hepatomegaly, but showed neither clinical muscle involvement nor hypoglycemia. Follow-up of patient 1 is under way.

p.W1327X is a prevalent mutation in Turkish GSD III patients. All six patients come from two nearby cities on the East Black Sea. AGL haplotype analysis showed that all six patients had the same haplotype, indicating a founder effect, that is, they had a common ancestor. As p.W1327X was reported in a Tunisian,²⁴ an Egyptian,¹⁹ a Caucasian from Canada¹⁶ and a German–Ukraine²⁵ patient, we compared AGL haplotypes of Egyptian, Caucasian and Turkish patients studied by us to determine whether the mutation occurred independently. Three haplotypes were different: (1) the Egyptian had t in rs2307129, whereas Turkish and Caucasian had c; (2) the Caucasian had a in rs1541041, whereas Turkish and Egyptian had t. These results suggested that W1327X is a recurrent mutation in various ethnic groups.

As for clinical features, we could not observe robust phenotype–genotype relationship. Even in patients with the same mutation, clinical features varied. For example, patients with p.W1327X had unique clinical courses. Patient 12 had macroglossia inhibiting her ability to speak at diagnosis. After the onset of dietary treatment with high protein, the symptom dissipated. As she was the eldest patient in the group, this problem may be associated with late diagnosis at an older age. Patient 16 had severe muscle weakness and died from cardiac failure at the age of 19 years, whereas patient 12 lives to the age of 55 and with mild muscle involvement. Other factors modulating the progression of clinical features may exist and further studies will be needed to elucidate them.

In summary, we showed the first comprehensive overview of clinical and molecular features of Turkish GSD III patients. This is the first report of the missense mutation being associated with isolated glucosidase deficiency.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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