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Mutational analysis of ten Turkish patients with glycogen storage disease type III: identification of four novel mutations

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Summary

Aim: *AGL* gene mutations are responsible for glycogen storage disease type III which is an autosomal recessive disorder. The distribution of these mutations shows a great variance in different populations. The aim of this study is to uncover the *AGL* gene mutation profile among Turkish patients and to contribute to the establishment of a link between these mutations and the clinical picture of the disease.

Material and Method: A total of ten patients aged between two and eight years (mean age 1.7+1.1) who were diagnosed with glycogen storage disease type III by liver biopsy and enzymatic analysis from eight different families were included in this study. DNA was isolated from the peripheral blood samples of these patients and exons 6, 7, 9-18, 22, 24, 29-34 of the *AGL* gene were studied by DNA sequencing analysis.

Results: Our study revealed two novel missense mutations p.G167V and p.Y173F, two novel intronic single base substitutions c.1284-1G>A and c.2002-2A>T and a known single base substitution p.W1327X. Numerous intronic variants were also identified. As a result of the analysis of ten patients, SNP's rs3736296, IVS12-197T>G, rs2291637, rs2035961, rs2274570, rs6692695, rs296885 were found in 1, 6, 1, 1, 1, 1, and 2 of the 10 patients, respectively.

Conclusions: According to the recent literature about the *AGL* gene which is constituted of a total of 35 coding exons, mutations have been reported frequently in exons 3, 4, 7, 16, 21, 25, 30 and 31. This study and previous studies reveal that the majority of the mutations identified in Turkish patients so far have been detected in exon 31 of the AGL gene. In addition, the distribution of *AGL* gene mutations in Turkish patients reflects the genetic heterogeneity in our population. *(Turk Arch Ped 2012; 47: 274-278)*

Key words: *AGL* gene, glycogen storage disease type III, mutation

Introduction

Glycogen debranching enzyme gene (AGL) codes 1,4-lpha-D-glucan:1,4-alpha-D-glucan4-alpha-D-glucosyltransferase which is a multifunctional enzyme involved in glycogen destruction and amilo-1,6-glucosidase (1). The mutations which occur in this gene have been associated with glycogen storage disease type III (GSD III, MIM#232400). While the group which constitutes approximately 85% of glycogen storage disease III patients and shows both hepatic and muscle involvement is called GSD IIIa, patients with only hepatic involvement constitute GSD IIIb group. Patients who exhibit loss of glycosidase enzyme activity alone are classified as GSD IIIc and patients who exhibit loss of transferase enzyme activity alone are classified as GDS IIId (2).

IVS32-12A>G mutation in AGL gene was defined in a Japanese GSD IIIb patient for the first time and studies conducted later showed the presence of very different types of mutations (2,3,4,5,6,7,8,9). Although most mutations have been reported only for once, at least four different recurrent mutations have been found in AGL gene (5,10,11,12,13). In addition, some mutations have been found to show geographical variation (14). Recently, a literature review reported 112 different mutations in AGL gene (15).

In this study, it was aimed to determine the changes in AGL gene in 10 Turkish patients with a diagnosis of GSD III and establish a genotype-pheotype relationship by evaluating clinical and biochemical findings in these patients.

Material and Method

Patients

A diagnosis of glycogen storage disease was made as a result of histopathologic examination by liver needle biopsy in

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the patients who were being followed up in Akdeniz University Medical Faculty, Division of Pediatric Gastroenterology, Hepatology and Nutrition. Enzymatic analyses of these patients were performed in Holland-Erasmus University-Rotterdam Clinic Genetic Laboratory and 10 patients who were diagnosed as GSD III were included in the study. Signed informed consent was obtained from all patients. The age of the patients ranged between two and eight (Mean age: 1,7 +1,1) and the female/male ratio was found to be 6/4. The first three patients were siblings. 40% of the patients presented with symptomatic hypoglycemia, 33% presented with abdominal distention and 20% presented with hepatomegaly (Table 1). Symptomatic hypoglycemia was found in 80% of the patients and gradual improvement with diet (nightlong frequent continuous gastric feeding with high-carbohydrate diet, frequent feeding and support with uncooked cornstarch at daytime) occured in 75% of these patients. Muscle weakness was observed in 50% of the patients. An increase in the frequency of otitis was not found in these patients compared to outpatients.

Creatinine kinase (CK) values were tested only in three patients at presentation and later measurement was done in all patients.

Echocardiogram was performed in seven patients and cardiomyopathy was found only in patient 8. Other patients had normal echocardiogram.

No significant change was found in lipid, uric acid, erythrocyte sedimentation rate, total protein and albumin values of the patients.

Molecular analyses

DNA was obtained from the peripheral blood samples of the patients using modified salt precipitation process (16). Certain regions of the amylo-alpha-1,6 glucosidase, 4-alpha-glucanotransferase (AGL) gene were screened in terms of mutations. Exons with number 6,7,9-18,22,24,29-34 (exon numbering was done by reference (1)) and related exon-intron linkage regions were copied by PCR using primary sequences in a previously performed study (6). Polymerase chain reaction products were moved in 2% agarose gel and viewed in UV using Genius Syngene Bio imaging device. In purification of polymerase chain reaction products, "High Pure PCR Product Purification Kit" (Trademark:Roche, Catalog number: 11 732 676 001) was used. Sequence analysis reaction was performed using fluorescent-based dye transfer inhibition system (Big Dye ver.1,1, Applied Biosystems [ABI], Foster City, CA). Capillary electrophoresis was applied to sequence analysis samples using ABI Prism 310 (PE biosystems, Foster City, CA) device in accordance with the instructions of the manufacturer. The results were evaluated using the AGL gene sequence (NG_12865,1, NM_000028,2, NP_000019,2) in NCBI (U.S. National Center for Biotechnology Information) database as reference. In definition of mutations, Human Genome Variation Society (HGVS) nomenclature was used.

Results

Hypoglycemia or short stature (as a long-term complication of glycogen storage) was observed in six of our ten patients (patient 1, 2, 3, 5, 8 and 9). Exercise intolerance and muscle weakness were observed in five of our ten patients and cardiomyopathy was observed in one of our ten patients (patient 8).

High-degree enlargement was found in the hepatocytes with glycogen accumulation (diastase labile, "periodic acid-Schiff positive). Hepatic steatosis, increased periportal fibrous tissue or cirrhosis was not observed in any patient.

Creatinine kinase levels were found to be increased at presentation in three of four patients and later increased CK levels were found in nine of ten patients. Since all CK values at presentation were not available, increased CK levels were determined later. However, CK level was normal in only one of six children who were older than 10 years and all the others had increased CK levels (mean: 510±305 U/L). In three of four children who were younger than 10 years, final CK levels were found to be higher than normal. Hepatic function tests were found to be normal in patient 1 who was 17 years old and in patient 6 who was 18 years old (values: 71/94 and 41/58, respectively). Interestingly, high CK level (810 U/L) and myopathy were found in patient 6 in contrast to patient 1. Transaminases were found to be 3-20 fold higher than normal in all patients at presentation, but final transaminase levels in patient 1 and 6 who were 17 and 18 years old, respectively were found to be normal.

All missense mutations, insignificant mutations and potential intronic mutations which may affect RNA function determined as a result of molecular analysis are shown in Table 1. p.G167V mutation which is a missense mutation defined in this study for the first time was found in two siblings and in another patient heterozygously (patient 1, 3 and 9). p.Y173F mutation which is another novel missense mutation was defined in one of the sibling patients (patient 3) heterozygously. Two different intronic region changes which may affect RNA function included heterozygous c.1284 -1 G>A in patient 5 and homozygous c.2002 -2 A>T in patient 9. These two changes were also defined in this study for the first time. p.W1327X mutation which was defined in previous studies and which causes formation of "inhibitor" codon was found in patient 7 homozygously.

In addition, c.471C>T (p.H1372H) mutation which is a silent mutation was found in patient 8 and 10 homozygously. Additionally, multiple intronic variants were found. While c.1424 -197 T>G (g.32362 T>G, IVS12-197 T>G) mutation was observed in six of ten patients studied, c.2001 +8 T>C (g.36092 T>C, rs3736296, IVS16+8 T>C), c.1424 -125 T>A (g.23434 T>A, rs2291637, IVS12-125 T>A), c.2950 -21 T>A (g.46502 T>A, rs2035961, IVS23-21 T>A), c.3836 +45G>A (g.65809 G>A, rs2274570, IVS29+45G>A), c.3949 +294 A>T (g.67728 A>T, rs6692695, IVS30+294A>T) and c.4260 -97 G>A (g.71230 G>A, rs2296885, IVS32-97G>A) mutations were found only in one patient each.

Table 1. Patient characteristics and genotypes

Patient number	Age at onset of disease	Final age at ollow-up/Gender	Consanguineous marriage	Reason for referral	ALT/AST (U/L) Value at presentation	ALT/AST (U/L) Value at the final follow-up vizit	Genotype	
1**	1	17/M	+	Symptomatic hypoglycemia, abdominal distention	670/1210	71/94	*g.19342G>T (exon 6) c.500G>T; p.G167V	Heterozygous
2**	2	2/F	+	Hepatomegaly	788/1621	646/1529	-	
3**	2	13/M	+	Symptomatic hypoglycemia	749/812	124/129	*g.19342G>T (exon 6) c.500G>T; p.G167V *g.19360A>T (exon 6) c.518A>T; p. Y173F	Combined Heterozygous
4	1	10/M	+	Symptomatic hypoglycemia, Hepatomegaly	132/181	343/323	-	
5	1	4/F	-	jaundice, abdominal distention	153/217	259/315	*g.31374G>A (intron 11) c.1284-1G>A	Heterozygous
6	2	18/F	-	Symptomatic hypoglycemia	124/168	41/58	-	
7	1	11/M	-	Hepatomegaly	265/474	137/178	***g.68474G>A (exon 31) c.3980G>A; p.W1327X	Homozygous
8	11	12/F	-	Symptomatic hypoglycemia, abdominal distention	471/251	156/176	-	
9	3	4/M	-	Abdominal distention	392/743	179/252	*g.19342G>T (exon 6) c.500G>T p.G167V *g.36207A>T (intron 16) c.2002-2A>T	Heterozygous Homozygous
10	3	8/M	+	Symptomatic hypoglycemia, abdominal distention	692/997	161/173	-	

Reference sequences: NG_12865.1, NM_000028.2, NP_000019.2. Reference number (1) was used in exon denomination. Mutations denoted with "*" were defined in this study for the first time.
**Patients 1, 2 and 3 are siblings. ***mutations which were defined previously

Discussion

In this study, AGL gene mutation results of 10 Turkish patients who were diagnosed as GSD III were evaluated together with clinical and biochemical findings. Similar to previous studies, no correlation was found between muscle involvement and CK levels (17,18). It is thought that CK levels should be measured again in the follow-up.

p.G167Y mutation which was defined in this study for the first time as a result of partial screening of the AGL gene was observed in patients 1, 3 and 9. Patient 1 and 3 are siblings and patient 9 is from another family. This mutation which was defined in our study for the first time is thought to lead to an amino acid change at protein level. However, the fact that p.G167Y mutation was found in only two of three siblings who had a diagnosis of GSD III and presence of homozygous c.2002 -2 A>T mutation in addition to p.G167Y mutation in patient 9 show that this mutation can not be associated with the disease. On the other hand, the fact that c.2002 -2 A>T mutation is only two bases away from the region of RNA processing shows that this mutation can cause abnormal protein formation by affecting RNA processing and this genotype can be associated with the phenotype. Heterozygous c.1284 -1 G>A mutation found in patient 5 was also defined for the first time in this study. We guess that this mutation may also affect protein structure by changing RNA processing. However, the other mutant allele needs to be investigated in this patient.

p.W1327X which is a known missense mutation was found homozygously in patient 7 who presented with hepatomegaly at the age of one. This mutation was previously reported in a family from the Mediteranean region (7), in a family from Egypt (19), in a caucasian family from Canada (13), in a family from Germany-Ukraina (10) and in five families from the Black Sea region of Turkey (12). These findings show that p.W1327X mutation is observed in different populations and may be one of the dominant mutations for the Turkish population considering that there was no consanguinity between the mother and father of patient 7.

At least 11 different mutations have been reported in Turkish GSD III patients in previous studies. In two Turkish families, IVS7 +5 G>A and IVS21+5ins A mutations were found (13). In a recent study, nine different mutations including p.R1147G, p.W373X, p.R595X, p.Q1205X, p.Q667X, p.Q1376X, c.293+2T>C, p.W1327X and c.1019delA have been determined (12). c.2474delC which is a novel mutation was reported in a Turkish GSD III patient (21). At least four mutations in the AGL gene have come to the forefront as the most common mutations in studies performed until the present time. These include IVS32-12 A>G, p.R408X, p.W1327X and c.750-753delAGAC mutations (5,10-13). Among these mutations, only p.W1327X mutation was found in the Turkish GSD III patients. This mutation was found in one of ten patients homozygously. Including this patient the mutations found in our three patients are thought to have a definite association with the clinical findings of GSD III. In addition, lack of observation of genetic changes with which this relationship can be established in the remaining seven patients does not exclude potential mutations in regions of the AGL gene which were not screened in our study. Conclusively, evaluation of all these findings in association shows that the clinical variation observed in the Turkish patients may be explained by allelic difference.

In addition, it was shown that mutations in exons 3, 4, 7, 16, 21, 25, 30 and 31 of the AGL gene were defined in at least three or more patients in an overall review performed in 2010 (15). Among 29 Turkish GSD III patients, one of two mutations (p.Q1376X and p.W1327X) in exon 31was found in 10 patients (12,13,21). As a result of these findings exon 31 is included in approximately 1/3 of the mutations found in the Turkish patients until the present time. Therefore, we think that exon 31 may be the “hot spot” region and should be primarily screened for mutation.

Since mutation screening studies is a noninvasive method, they are very useful in making the diagnosis. We think that the genotype-phenotype relationship can be established better by screening more exons of the AGL gene and more patients in GSD III which shows both genetic and clinical variance in our population as in some other populations.

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Conflict of interest: None declared.

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