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ORIGINAL ARTICLE

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Molecular characterization of Egyptian patients with glycogen storage disease type IIIa

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Abstract Glycogen storage disease type IIIa (GSD IIIa) is an autosomal recessive disorder characterized by excessive accumulation of abnormal glycogen in the liver and muscles and caused by a deficiency in the glycogen debranching enzyme. The spectrum of *AGL* mutations in GSD IIIa patients depends on ethnic group—prevalent mutations have been reported in the North African Jewish population and in an isolate such as the Faroe islands, because of the founder effect, whereas heterogeneous mutations are responsible for the pathogenesis in Japanese patients. To shed light on molecular characteristics in Egypt, where high rate of consanguinity and large family size increase the frequency of recessive genetic diseases, we have examined three unrelated patients from the same area in Egypt. We identified three different individual *AGL* mutations; of these, two are novel deletions [4-bp deletion (750–753delAGAC) and 1-bp deletion (2673delT)] and one the nonsense mutation (W1327X) previously reported. All are predicted to lead to premature termination, which completely abolishes enzyme activity. Three consanguineous patients are homozygotes for their individual mutations. Haplotype analysis of mutant *AGL* alleles showed that each mutation was located on a different haplotype. Our results indicate the allelic heterogeneity of the *AGL* mutation in Egypt. This is the first report of *AGL* mutations in the Egyptian population.

Keywords Glycogen storage disease type III · *AGL* · Deletion · Nonsense mutation · Haplotype · Egypt

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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 (Chen 2001). GSD III is caused by a deficiency in the glycogen debranching enzyme, a key enzyme in the degradation of glycogen. The enzyme has two independent catalytic activities, oligo-1,4-1,4-glucantransferase (EC 2.4.1.25) and amylo-1,6-glucosidase (EC 3.2.1.33), on a single 160-kDa protein. The glycogen-binding site is assumed to be located in the carboxyl terminal of its protein. Both activities and glycogen binding are required for complete function. In GSD III patients, enzyme activities are virtually absent in affected organs. Most patients have both liver and muscle involvement (GSD IIIa), but approximately 15% of the patients have solely liver involvement without any muscular manifestations (GSD IIIb). These subtypes have been explained by differences in tissue expression of the deficient enzyme.

The human *AGL* gene (*AGL*) has been isolated and shown to be 85 kb in length and composed of 35 exons, encoding a 7.0-kb mRNA (Bao et al. 1996). Multiple tissue-specific isoforms of *AGL* mRNAs, differing only at the 5′- end, have been reported, and the predominant form, liver glycogen debranching enzyme, is predicted to have 1,532 amino acids, deduced from mRNA isoform 1

(Bao et al. 1997). Molecular analyses of GSD IIIa have been performed in Caucasian, Japanese, Italian, Jewish, and several other ethnic populations (Shen and Chen 2002) and over 50 different *AGL* mutations have been reported in GSD III patients (Human Gene Mutation Database; http://www.hgmd.org).

The spectrum of *AGL* mutations in GSD IIIa varies among ethnic groups. In an ethnic group with a high rate of consanguinity, prevalent mutations have been reported. For example, in the North African Jewish population, a single *AGL* mutation (4455delT) is prevalent (Parvari et al. 1997). In the Faroe islands, a small archipelago in the North Atlantic and an isolate, one specific mutation (R408X) is responsible for GSD IIIa patients (Santer et al. 2001). Information on prevalent mutations helps facilitate DNA-based diagnosis of GSD III in these specific populations.

In contrast, genetic heterogeneity has been shown in other ethnic groups. We have reported the heterogeneity of *AGL* mutations in Japan. Eleven different mutations have been identified in our studies (Horinishi et al. 2002; Okubo et al. 1996, 1998, 1999, 2000a, b). Lucchiari et al. (2002) discovered genetic heterogeneity in Italian patients also, although one splicing mutation (IVS21 + 1G > A) accounts for 28%. In Caucasian populations four mutations make up approximately 28% of all GSD IIIa patients, but the rest of the mutations are heterogeneous (Shen and Chen 2002). These findings show that the spectrum of *AGL* mutations in GSD IIIa depends on ethnic group.

In Egypt, Arab is the major ethnic group. The Arabs do not usually prohibit marriages with relatives, and consanguinity rates range between 20 and 60% (Teebi et al. 2002). Moreover, families have an average of 5.3 children in Egypt. Both high rates of consanguinity and large family size increase the frequency of autosomal recessive genetic diseases. These factors suggested to us that a prevalent mutation might be found in Egyptian GSD IIIa patients. To address this question, we have examined three unrelated patients from the same area in Egypt. We identified three different mutations in the three patients and observed allelic heterogeneity of GSD IIIa in Egypt. This is the first report of *AGL* mutations in the Egyptian population.

Materials and methods

Patients

Three Egyptian GSD IIIa patients from three unrelated families were investigated. They were from the delta region in Egypt (Fig. 1). The patients were confirmed as having deficient debranching enzyme activity in peripheral red blood cells by the method of Shin (1990). All patients showed both liver and muscle involvement and were diagnosed with GSD IIIa. Consanguinity was ascertained in all families. The study was approved by the local ethics committees and performed with the patients' and their families' informed consent.

Fig. 1 Geographical origin of patients with glycogen storage disease type IIIa in Egypt. The three patients are from the delta region, indicated by an *arrow*

DNA sequence analysis of the *AGL* gene

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* genes were sequenced directly as described previously (Okubo et al. 2000b). The nucleotides of *AGL* cDNA were numbered according to *AGL* isoform 1 (GenBank accession no. NM_000642).

Mutation analysis of the *AGL* gene

Point mutations identified in patients were verified using restriction fragment length polymorphism (RFLP). A pair of primers (listed in Table 1) was used for PCR and each specific-restriction endonuclease was added to digest PCR products. Restriction digests were analyzed on polyacrylamide gel. Fifty-five Japanese control subjects were examined by RFLP in the same manner to eliminate the possibility they are mere polymorphisms in controls.

Haplotype determination in the *AGL* gene

Twenty-three polymorphic markers in the *AGL* gene were genotyped in accordance with previous reports (Horinishi et al. 2000, 2002; Okubo et al. 2000b; Shen et al. 1997).

Table 1 PCR primers using RFLP detection for *AGL* mutations

Location	Mutation	PCR primer (5′ → 3′) for RFLP detection	Restriction enzyme	Fragment size (bp)
Exon 7	750–753delAGAC	F: ctt cag CTG CTA ATA GTA AAT GG R: CAT CAC AGG AGA AAC GCC AAA GAC CTC T	*Psh*AI	Normal: 103 + 26 Mutant: 125
Exon 21	2673delT	F: CAA TGC AGA TCC TAT ATT AAA AGC ACC T R: ctg ctt aat tca gtc tag tgg	*Bst*API	Normal: 107 Mutant: 77 + 29
Exon 31	3980G > A [W1327X]	F: AAG GCT ATA AAG GTC TCA TAT GAT GTC T R: CCA GAT TTG GAT GCT TTT CAT	*Xba*I	Normal:115 Mutant: 89 + 26

Exon sequences are represented by uppercase letters and introns by lowercase letters. Mismatch nucleotides are underlined
F forward, *R* reverse

Results

We identified three different *AGL* mutations in the three unrelated Egyptian patients.

Sequence analysis for patient 1 revealed a 4-bp deletion from nucleotides 750–753 (750–753delAGAC) in exon 7 (Fig. 2). Deletion of 4 bp results in a frame shift, leading to premature termination at codon 273. RFLP

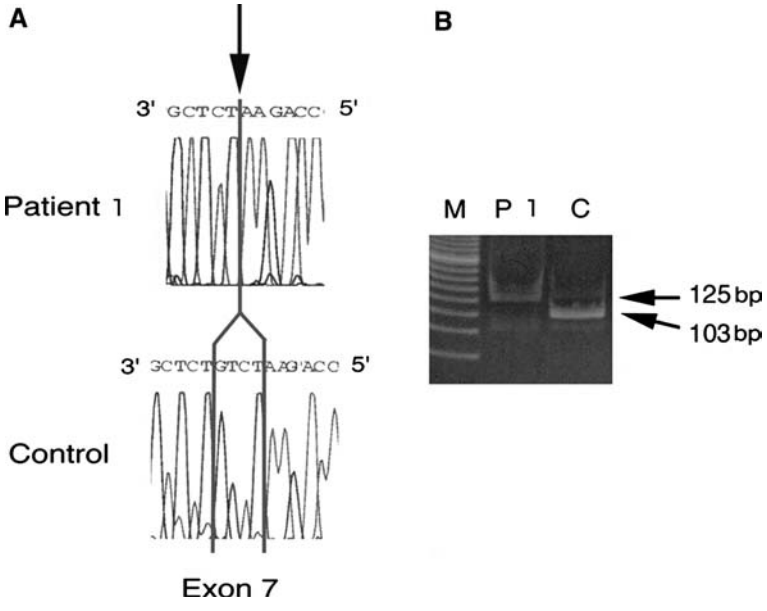

Fig. 2 Mutational analysis for patient 1. **A**. In the *upper panel* the sequence electropherogram of the antisense strand corresponding to the 4-bp deletion in exon 7 of the AGL gene is shown. The point of the deletion is indicated by an *arrow*. Patient 1 was homozygous for the 4-bp deletion. Normal sequence from a control is shown in the *lower panel*. **B**. Electrophoretic analysis of PCR products after digestion with *Psh* AI. *M*, DNA marker; *P1*, patient 1; *C*, control. Patient 1 had a 125-bp fragment alone, verifying homozygosity for the 750-753delAGAC mutation

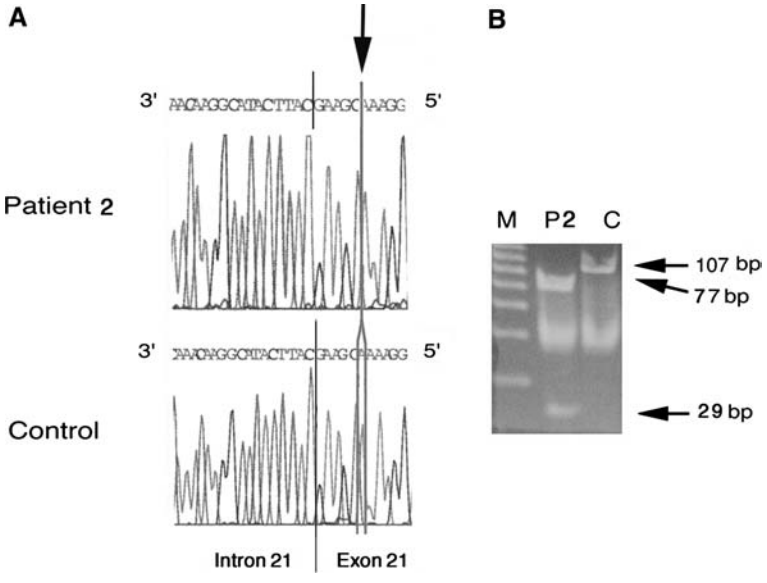

Fig. 3 Mutational analysis for patient 2. **A**. In the *upper panel* the sequence electropherogram of the antisense strand corresponding to the 1-bp deletion in exon 21 is shown. The point where the deletion occurred is indicated by an *arrow*. Patient 2 was homozygous for the 1-bp deletion. Normal sequence from a control is shown in the *lower panel*. **B**. Electrophoretic analysis of PCR products after digestion with *Bst* PAI. *M*, DNA marker; *P2*, patient 2; *C*, control. Patient 2 had 77-bp and 29-bp fragments, verifying homozygosity for the 2673delT mutation

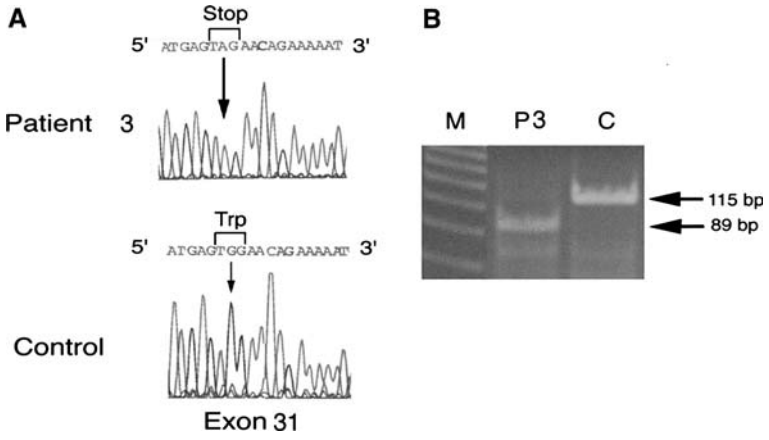

Fig. 4 Mutational analysis for patient 3. **A**. In the *upper panel* the sequence electropherogram of the sense strand in exon 31 is shown. A G-to-A substitution at nucleotide 3980 in patient 3 is indicated by an *arrow*. Patient 3 was homozygous for the nonsense mutation (W1327X). Normal sequence from a control is shown in the *lower panel*. **B**. Electrophoretic analysis of PCR products after digestion with *Eco* RI. *M*, DNA marker; *P3*, patient 3; *C*, control. Patient 3 had an 89-bp fragment alone, verifying homozygosity for the W1327X mutation

analysis with restriction enzyme *Psh*AI confirmed that patient 1 was homozygous for the 4-bp deletion.

Patient 2 had a 1-bp deletion of nucleotide 2673 (2673delT) in exon 21 (Fig. 3). Deletion of 1 bp causes premature termination at codon 899, because of a frame shift. RFLP analysis with restriction enzyme *Bst*PAI verified that patient 2 was homozygous for the 1-bp deletion.

Sequence analysis for patient 3 revealed a G-to-A substitution at nucleotide 3980 in exon 31 that replaces tryptophan by termination at codon 1327 (W1327X) (Fig. 4). RFLP analysis with *Xba*I indicated that patient 3 was homozygous for W1327X.

These three mutations were not found in the 55 normal controls.

Haplotype analysis of three mutant alleles demonstrated that each mutation was located on a different *AGL* haplotype (Table 2).

Table 2 Haplotyping of mutant *AGL* alleles

Mutation Polymorphism	Patient 1 750–753delAGAC	Patient 2 2673delT	Patient 3 W1327X
−787	**aa**	gg	**aa**
−10 in exon 3	**gg**	aa	aa
IVS3 + 85	**tt**	**tt**	cc
−2 in exon 4′	**cc**	**cc**	tt
IVS4-122	**tt**	**tt**	**tt**
IVS6-73	gg	**aa**	**aa**
298L	**tt**	**tt**	cc
R387Q	**RR**	**RR**	**RR**
IVS12 + 74	tt	tt	**cc**
IVS12-125	aa	aa	**tt**
IVS13-70	gg	gg	**cc**
IVS16 + 8	cc	cc	**tt**
IVS21 + 124	**aa**	**aa**	gg
IVS22 + 11	**aa**	**aa**	gg
IVS23-121	gg	gg	**aa**
IVS23-21	aa	aa	**tt**
P1067S	**cc**	**cc**	**cc**
G1115R	**GG**	**GG**	**GG**
R1253H	**RR**	**RR**	**RR**
IVS29 + 45	**aa**	gg	gg
IVS29 + 53	**aa**	tt	tt
E1343K	**EE**	**EE**	**EE**
IVS32-97	**aa**	gg	gg

Major alleles are emboldened in accordance with our data on Japanese controls

Discussion

We have shown the allelic heterogeneity of the *AGL* mutation in Egypt. Our genetic analysis of three Egyptian patients from the same area revealed three different mutations. Furthermore, haplotype analysis revealed that each mutation was located on a different *AGL* haplotype. These findings suggest that no founder mutations are present in the Egyptian population and that heterogeneous mutations are responsible for the disease in Egyptian GSD IIIa patients. We are trying to recruit more GSD IIIa patients to confirm our finding.

We have revealed three different *AGL* mutations for individual families. Patient 1 is homozygous for 750–753delAGAC and patient 2 is homozygous for 2673delT. Both small deletions are predicted to lead to truncated proteins lacking the glycogen-binding site in the carboxyl terminal, because of frame shift. These are novel *AGL* mutations that have not previously been reported. Patient 3 is a homozygote for W1327X. All are predicted to lead to premature termination, which completely abolishes enzyme activity. These premature stop codons will probably be recognized as nonsense-mediated decay, leading to the absence of *AGL* mRNA. These results are consistent with the finding that most of the mutations reported thus far are non-

sense mutations caused by a nucleotide substitution, deletion, or insertion (Shen and Chen 2002).

As far as we are aware patient 3 is the second patient with the W1327X mutation. Lucchiari et al. (2002) has reported W1327X in a Tunisian GSD III patient. We could not discover whether or not W1327X is a recurrent mutation, because no information on the haplotype of the Tunisian patient was given in Lucchiari's study. The two patients could share a common ancestor, because Egypt and Tunisia are geographically near, located in North Africa, and the main ethnic group in both countries is Arab.

In summary, we identified three different *AGL* mutations in Egyptian GSD IIIa patients, showing that molecular defects of *AGL* were heterogeneous in Egypt. This is the first report of *AGL* mutations in Egyptian patients.

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