High frequency of W1327X mutation in glycogen storage disease type III patients from central Tunisia

Abstract. Glycogen storage disease type III (GSD III) is an autosomal recessive disorder caused by the deficiency of glycogen debranching enzyme (AGL). It is characterized by hepatomegaly, progressive myopathy, cardiomyopathy and fasting hypoglycemia. Several mutations in AGL gene have been described in different populations. The W1327X mutation was reported in one Tunisian patient resident in Italy. We looked in this report to determine the frequency of W1327X mutation among Tunisian patients. The W1327X mutation was screening in 26 GSD III patients originated from various geographic locations in Tunisia. The sequence analysis revealed that among nine patients carried the W1327X mutation. Eight of them were from six unrelated families and they were originated from Mahdia (centre of Tunisia) suggesting the existence of a founder effect in this region. Taking into account historical migratory waves, screening for this mutation should be performed in priority for molecular diagnosis confirmation of GSD III in North African populations.

Key words: glycogen storage disease type III, molecular diagnosis, Tunisia, founder effect, W1327X mutation

Résumé. La glycogénose de type III (GSD III) est une maladie autosomique récessive due à un déficit de l’enzyme débranchante du glycogène (AGL). Elle est caractérisée par une hépatomégalie, une myopathie progressive, une cardiomyopathie et une hypoglycémie à jeun. Plusieurs mutations ont été décrites au niveau du gène AGL dans différentes populations. La mutation W1327X a été reportée chez un patient tunisien qui réside en Italie. Dans ce travail, nous avons cherché à déterminer la fréquence de cette mutation chez les patients tunisiens. La mutation W1327X a été dépistée chez 26 patients atteints de GSD III. Nos résultats révèlent que parmi neuf patients porteurs de la mutation W1327X, huit sont originaires de Mahdia (centre de la Tunisie), suggérant l’existence d’un effet fondateur dans cette région. En tenant compte de l’historique des flux migratoires, la recherche de cette mutation devrait être effectuée en priorité pour le diagnostic moléculaire de la GSD III chez les patients originaires d’Afrique du Nord.

Mots clés : glycogénose de type III, diagnostic moléculaire, Tunisie, mutation W1327X
Glycogen storage disease type III (GSD III, OMIM 232400) is an autosomal recessive disorder characterized by fasting hypoglycemia, short stature, hepatomegaly, progressive myopathy, and cardiomyopathy. Most patients have both liver and muscle involvement (GSD IIIa), but approximately 15% of patients have only liver involvement without any muscular manifestations (GSD IIIb) [1]. GSD III is caused by the deficiency of glycogen debranching enzyme (AGL). This enzyme plays an important role in the degradation of glycogen and has two catalytic activities: an oligo-1,4,1,4-glucanotransferase (EC 2.4.1.25) and an amylo-1,6-glucosidase (EC 3.2.1.33) at two separate sites on a single 160 kDa protein. Both activities are required for an appropriate function. The glycogen-binding site is assumed to be located in the carboxyl terminal of the protein. The deficiency of AGL causes excessive accumulation of abnormal glycogen with truncated outer chains [2].

The gene encoding for AGL is located on 1p21. It contains 35 exons and spans approximately 85 Kb. Six mRNA isoforms that differ in their 5' untranslated region have been reported. They result from differential transcription and alternative exon splicing. The predominant isoform is predicted to have 1,532 amino acids, encoded from the mRNA isoform 1 [3]. So far, more than 50 mutations have been reported in the literature (www.hgmd.org).

Previous studies have revealed that the spectrum of AGL mutations depends on ethnic background. Indeed, molecular analyses reported in Jewish, Japanese, Caucasian and Italian populations revealed a specific population spectrum of AGL mutations in GSD III [4]. In 2002, Lucchiari et al. were the first to describe the W1327X mutation in a Tunisian GSD III patient [5]. This mutation seems to be the third recurrent mutation reported in the AGL gene [6].

The screening of W1327X mutation in Tunisians GSD III patients have been carried, we report here our preliminary findings.

**Materials and methods**

**Subjects**

Twenty-six patients (12 females and 14 males) from twenty-three unrelated families originated from various geographic locations in Tunisia were investigated. The diagnosis of GSD IIIa was based on typical clinical and laboratory finding such as hepatomegaly, recurrent hypoglycaemia, hyperuricemia. The diagnosis was confirmed by measurement of enzyme activity only in eleven patients. For the other patients both clinical and biochemical manifestations were highly suggestive of GSD III.

**Mutation analysis**

Genomic DNA of each proband and their both parents was extracted from peripheral blood leukocytes by classical phenol/chloroform extraction or salting out method. The exon 31 of AGL gene was amplified from genomic DNA. The PCR products were purified by use of the QIA quick gel extraction purification kit (Qiagen) and sequenced with the Big Dye terminator kit (Applied Biosystems, Foster City, CA, USA) using PCR primers on an ABI prism 377 or 3130 DNA sequencer (Applied Biosystems) in accordance with the manufacturer’s recommendations.

**Results**

The sequence analysis revealed that among twenty six Tunisians patients, eight were homozygous for W1327X mutation and one was compound heterozygous (figure 1). The majority of Tunisian patients carrying the W1327X mutation originated from Mahdia region in centre of Tunisia (eight patients from six unrelated families).

**Discussion**

In Tunisia, the prevalence of GSD III remains unknown. Given the high rate of consanguinity in our country the GSD III is likely more frequent than reported in other countries. At the exception of a study on Egyptian patients [6], so far mutation spectrum of GSD III in North Africa is unknown. The W1327X mutation has been the first described in Tunisian GSD III patient resident in Italy [5]. This mutation is a substitution of guanine (G) by adenine (A) at 4380 position in exon 31, this change is predicted to lead to premature termination and gives a truncated protein. Taking into account the relatively large size of AGL gene, we started our molecular study with the search for this mutation in twenty-six Tunisian patients (12 females and 14 males) from twenty-three unrelated families originating from various geographic locations in Tunisia. The sequence analysis revealed that among investigated patients, eight were homozygous and one was compound heterozygous. The majority of Tunisian patients that carried the W1327X mutation (eight patients from six unrelated families), are originated from center of Tunisia (Mahdia). As this mutation was also found in an Egyptian patient who originated from Delta region [5], a common ancestral allele between Tunisian and Egyptian patients could be explained by population migration flows during the Fatimide dynasty apogee. The W1327X mutation was also reported in a Caucasian patient from Canada [7], in a German-Ukraine patient [8] and in six Turkish patients [9]. In order to determine
whether this mutation had occurred independently, the authors compared the AGL haplotypes of the patients. The analysis revealed that the W1327X mutation was located on the same haplotype between Turkish patients and the Caucasian patient from Canada. While the Egyptian patient had a different haplotype. These findings suggest that the W1327X mutation seems to be a recurrent mutation [7].

Taking into account common genetic background due to historical migratory waves, the screening for this mutation should be performed in priority for molecular diagnosis confirmation of GSD III in North African populations. This situation is similar to other metabolic diseases such as Niemann Pick type B [10] and glycogenosis type I [11]. For these diseases, no biochemical diagnosis is available in North Africa. Molecular investigation provides an easier tool for diagnosis and circumvents from an invasive liver biopsy for confirmation of GSD.

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Conflicts of interest: none.

References


