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Biotinidase deficiency: Novel mutations in Algerian patients

A. Tiar [a,d,*], A. Mekki [b], M. Nagara [a,d], F. Ben Rhouma [a,d], O. Messaoud [a,d], N. Ben Halim [a,d], R. Kefi [a,d], M.T. Hamlaoui [c], A. Lebied [b], S. Abdelhak [a,d]

[a] Institut Pasteur de Tunis, Laboratoire de Génomique Biomédicale et Oncogénétique LR11IPT05, Tunis, 1002, Tunisia
[b] Pediatric Department, Hussein-Dey Hospital, Algiers, Algeria
[c] Pediatric Critical Care Unit, Hussein-Dey Hospital, Algiers, Algeria
[d] Universitè Tunis El Manar, Tunis, 1068,Tunisia

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ABSTRACT

Biotinidase deficiency is an autosomal recessive disorder of biotin metabolism leading to varying degrees of neurologic and cutaneous symptoms when untreated. In the present study, we report the clinical features and the molecular investigation of biotinidase deficiency in four unrelated consanguineous Algerian families including five patients with profound biotinidase deficiency and one child characterized as partial biotinidase deficiency. Mutation analysis revealed three novel mutations, c.del631C and c.1557T>G within exon 4 and c.324-325insTA in exon 3. Since newborn screening is not available in Algeria, cascade screening in affected families would be very helpful to identify at risk individuals.

1. Introduction

Biotinidase (EC3.5.1.12) is responsible for biotin recycling which is a coenzyme for four carboxylases in humans (Wolf, 2005). Biotinidase deficiency (BD, OMIM 253260) is an autosomal recessively inherited metabolic disorder (Wolf et al., 1983), characterized by seizures, hypotonia, skin rash, alopecia, ataxia, optic abnormalities, hearing loss and developmental delay with ketolactic acidosis and organic aciduria (Wolf et al., 1985). Based on the measure of biotinidase activity, individuals could be classified into two groups: profound biotinidase deficiency group (<10% of mean normal serum activity) and partial biotinidase deficiency group (10–30% of mean normal serum activity) (Hymes et al., 2001). The human biotinidase gene (*BTD* MIM: 609019) is localized in chromosome 3p25, consists of 4 exons with a total length of 1629 bp and encodes a mature protein of 543 amino acids resulting in a molecular mass of 56,771 Da (Knight et al., 1998). In 2007, Pindolia and collaborators predicted the putative three dimensional structure of biotinidase using homology to various related amidases and nitrilases (Pindolia et al., 2007). Currently, more than 100 mutations causing profound BD have been described (Pindolia et al., 2010). Genotype/phenotype correlations are not well established. Indeed only one study reported that children with symptoms of profound biotinidase deficiency with null mutations were more likely to develop hearing loss than those with missense mutations, even if not treated for a period of time (Sivri et al., 2007).

The disorder can be treated with oral administration of pharmacological doses of Biotin (Wolf, 2003). Newborn screening for BD is important and is conducted in many countries (Wolf, 1991). In Algeria, no clinical and epidemiological data are available and no molecular studies have been performed so far. In the present study, we report on the clinical and molecular investigation of BD in four unrelated Algerian families.

2. Materials and methods

2.1. Patients

We investigated four unrelated Algerian families, including a total of five symptomatic profound BD patients and one child, sibling of patients P1 and P2, characterized as partial BD patients by biochemical investigation. All families were consanguineous with a history of death caused by BD among siblings. Patients are from Kabylie, located in the north of Algeria with an Amazigh origin, the free people referred to by Europeans as “Berbers”.

Biotinyl-hydrolase activity was performed in France and determined semi quantitatively by a colorimetric assay that uses biotinyl-*p*-aminobenzoate as substrate and allowed to classify patients with either profound or partial BD. (Wolf and Secor McVoy, 1983).

After informed consent, blood samples were collected from BD patients and their available family members.

Abbreviations: IL-10, interleukin-10; DLBCL, diffuse large B-cell lymphoma; SNPs, single nucleotide polymorphisms; HWE, Hardy–Weinberg equilibrium; CI, confidence interval; OR, odds ratio; et al., et alia; NHL, non-Hodgkin's lymphoma; MAF, minor allele frequency; AS-PCR, allele-specific polymerase chain reaction; PCR-RFLP, polymerase chain reaction and restriction fragment length polymorphism.

* Corresponding author at: Laboratoire de Génomique Biomédicale et Oncogénétique, Institut Pasteur de Tunis, BP 74, 13 Place Pasteur 1002 Tunis Belvédère, Tunisia. Tel.: +216 71 849 110; fax: +216 71 791 833.
E-mail address: afaf.tiar@yahoo.fr (A. Tiar).

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2.2. PCR and direct sequencing

Genomic DNA was isolated from EDTA whole blood as previously described (Miller et al., 1988) and stored at −20 °C before analysis. The primer pairs and PCR reaction conditions that were used to amplify and sequence the 4 exons and exon-intron boundaries of *BTD* are listed in Table 1. For all PCR reactions, we used 30 ng of genomic DNA, 10× PCR buffer, 0.2 mM of each dNTP, 1.5 mM MgCl2, 0.5 mM of forward and reverse primers and 0.5 U Amplitaq DNA polymerase (Invitrogen, Foster City, CA, USA) in a total volume of 25 μl. Amplification was performed at an initial denaturation time of 94 °C for 5 min, followed by 35 cycles of 94 °C for 30 s, annealing temperatures for 30 s, and 72 °C for 1 min, with a final extension interval at 72 °C for 10 min. Purity and size of the PCR products were checked on a 1% agarose gel using standard electrophoresis methods. Sequencing was performed in forward and reverse directions using the Big Dye terminator kit (Applied Biosystems, Foster City, CA, USA) on an ABI prism 3130 DNA Genetic Analyzer (Applied Biosystems) according to the manufacturer's recommendations. DNA sequences were compared to the reference sequence from the NCBI Entrez Nucleotides Database (NM_000060.2).

3. Results

In this study, we report clinical and molecular investigation of BD in 5 Algerian patients. All patients presented characteristic clinical features of the disease. Only three patients (P1, P3 and P4) showed irreversible traits as hearing loss and developmental delay. Clinical information is summarized in Table 2. Sequencing analysis revealed three novel mutations in the investigated patients (Table 2; Fig. 1). The first mutation, detected in patients P1, P2 and P5 at a homozygous state, is a single nucleotide deletion (c.631delC) in exon 4. This deletion likely results in a frameshift and the production of a termination codon 53 amino acids downstream (p.Arg211ValfsX53). Individual P6 a sibling of patients P1 and P2, diagnosed neonatally by semi quantitative enzyme assay, showed partial BD. Molecular investigation revealed that this individual is heterozygous for the c.631delC deletion and complete sequencing of the *BTD* gene coding region did not reveal an additional mutation on the second allele.

The second mutation, a T to G transversion at position 1557 (c.1557T>G), was found in patient P3 at a homozygous state. The transversion should lead to a substitution of the tyrosin codon with a stop codon (p.Tyr519X) and creates a premature truncation of the BTD protein in the same exon. For patient P4, a homozygous insertion of two nucleotides TA in exon 3 (c.324–325insTA) was observed. The mutation is speculated to create a premature termination codon 109 amino acids downstream (p.Val109X). All parents were heterozygous for the respective mutations. Indeed Mendelian inheritance was confirmed in the families.

Table 1
Details of primers and PCR conditions for DNA amplification. Exon 4 was examined in two overlapping fragments (A + B).

Region amplified	Primer pair	Product size (pb)	Annealing temperature (C°)
Exon 1	5′CGGTCTAAATTCGTCCACT 5′GATTTAAGTAACGTGCGCT	553	53
Exon 2	5′CAGTATCACTGCGAGTGAGT 5′AGGTTAACTACCTGGATGCT	506	60
Exon 3	5′CAGAGTAACTTCCTGATGGT 5′CCTTGTAACGTCAGACATTC	444	56
Exon 4A	5′GGTGGTCTCAATCTCCTGAC 5′GTGGAGATAGCCTTCCTTTC	892	61
Exon 4B	5′GTTGATCAAGTGACACCCAG 5′CTGGGTGTCACTTGATCAAC	869	61

4. Discussion

BD is a rare recessive metabolic disease characterized by a clinical and a mutational heterogeneity. The worldwide prevalence of the disease is 1/60,000 (Wolf, 1991). In Algeria, no epidemiological data are available.

To our knowledge, the present study is the first reported clinical and molecular investigation of BD in Algeria. Three novel mutations were identified in the studied families: c.631delC, c.1557T>G and c.324–325insTA (Table 2).

The c.631delC mutation is flanked by short overlapping inverted and direct repeats (Fig. 1). These motifs favor the occurrence of deletion events (Charfeddine et al., 2003; Krawczak and Cooper, 1991). On the other hand, the insertion (c.324–325insTA) is likely due to a slipped mispairing mechanism mediated by a direct TA repeats (Fig. 1) (Cooper and Krawczak, 1991). Both of these mutations are conserved in mammals (Pindolia et al., 2007; Swango and Wolf, 2001) and are located in nitralase/amidase homologous domain that is essential for the enzyme activity (Pindolia et al., 2007). The p.Tyr519X nonsense mutation occurs in the carboxy-terminus of the enzyme, the site of several other mutations that cause profound deficiency (Pindolia et al., 2010). This shows the importance of the carboxy-terminus domain of biotinidase, although its function remains to be determined.

Clinical investigation showed inter- and intra-familial heterogeneity for the same pathogenic mutation as reported elsewhere (Wolf et al., 1983). All children developed dermatological and biochemical symptoms, whereas neurological traits were variable (Table 2). This could be associated with the age at diagnosis and age at biotin administration; therefore pediatricians should become more aware of the symptoms. Indeed, for family BD-I, although, P1 and P2 siblings share the same deleterious mutation, only patient P1 developed some neurological abnormalities, especially hearing loss while his brother P2 was asymptomatic.

Previous studies described that most profound BD children worldwide were found to be compound heterozygous, mainly in populations from Europe and America (Wolf, 2010). This high proportion of compound heterozygote affected individuals may be due to well-known increased urbanization, population admixture and isolate break-up (transition from a population consisting of reproductively isolated groups to a mixed population) (Campbell et al., 2009). In contrast all mutations identified among Greater Middle-Eastern populations like Saudi Arabia, Syria, Turkey and Morocco, in addition to the present study patients were in a homozygous pattern. The large number of homozygous mutation carriers in these countries is likely due to the high rate of consanguinity (Iqbal et al., 2010; Mikati et al., 2006; Pomponio et al., 2000a,b). Despite the fact that the four investigated families are from Kabylie, a limited geographic radius with a high ethnic endogamy (Hanouti, 1996), we identified three different new mutations reflecting an important mutational heterogeneity of BD. The finding that two unrelated families shared the same new mutation suggests that it could be a founder mutation specific to Kabylie. Nevertheless, this specificity must be taken with caution as more BD patients from other Algerian ethnic groups or from elsewhere in the world must be investigated (Romdhane et al., 2012). Implications of founder mutations in molecular investigation of rare orphan disease of genetic diseases have been discussed as a valuable molecular diagnostic tool especially in isolated populations such as in North Africa (Romdhane et al., 2012). Therefore, establishing the BD mutation spectrum with a focus on founder mutations could help improve molecular diagnosis of this disease as well as genetic counseling in Algeria allowing for earlier management, treatment, and follow-up.

Many countries perform newborn screening of BD (Wolf, 1991). In Algeria and North African countries, since newborn screening is not available, the cascade genetic screening could be performed for families at risk. This strategy is interesting not only for the implementation of