

## **Identification of a p.Cys33PhefsX36 mutation in an Iranian family with profound biotinidase deficiency (BTD)**

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### **Short Report**

Biotinidase deficiency (BTD; OMIM 609019) is one of the most treatable and an autosomal recessively inherited metabolic disorder in which the body is unable to reuse and recycle the vitamin biotin and resulting in multiple carboxylase deficiency.

The BTD gene is in charge for coding of biotinidase enzyme which is responsible to catalyze the cleavage of biotin from biocytin or biotinylated peptide and thereupon release of lysine and biotin (Wolf, Grier et al. 1983).

Profound or partial biotinidase deficiency worldwide prevalence is estimated at about 1 in 60,000 newborns and 1 in every 123 individuals is carrier for this disorder (Wolf and Heard 1990).

The clinical features of BTD typically appear within the first few months of life (usually between 2nd and 5th months), but the age of onset varies (from 1 week up to 10 years) (Wolf 2010).

BTD clinically presents progressive neurological deterioration (seizures, encephalopathy, neuro-developmental delay) associated with cutaneous involvement (skin rash, seborrhea, alopecia). Patients with

profound and partial biotinidase deficiency have less than 10% and 10-30% medial normal serum biotinidase activity respectively (Dobrowolski, Angeletti et al. 2003; Gonzalez, Marrero et al. 2006).

Despite the high prevalence of this disorder almost no genetic studies in order to find the mutation repertoire of this disorder in Iranian population have been conducted. Here we report the first mutation identified so far in an Iranian family with BTD.

A 3 months old girl, born to a first cousin marriage has been referred to our lab at the medical genetics department of Pars Hospital Lab in Tehran-Iran with seizures, feeding difficulty, weak muscle tone (hypotonia) and developmental delay. Concentration of 3-Hydroxyisovalerylcarnitine in her was elevated (4.6  $\mu\text{mol/L}$ ; reference range  $<0.5 \mu\text{mol/L}$ ). The ratio of 3-Hydroxyisovalerylcarnitine to acetylcarnitine was 0.18 and biotinidase activity was not detectable in her which was compatible with the diagnosis of profound BTD.

Mutations in the *BTD* gene cause biotinidase deficiency. In order to confirm the diagnosis of BTD, DNAs were extracted from the affected girl as well as her parents.

The entire coding region and the highly conserved exon-intron splice junction of *BTD* gene were screened by PCR amplification and direct sequencing of both

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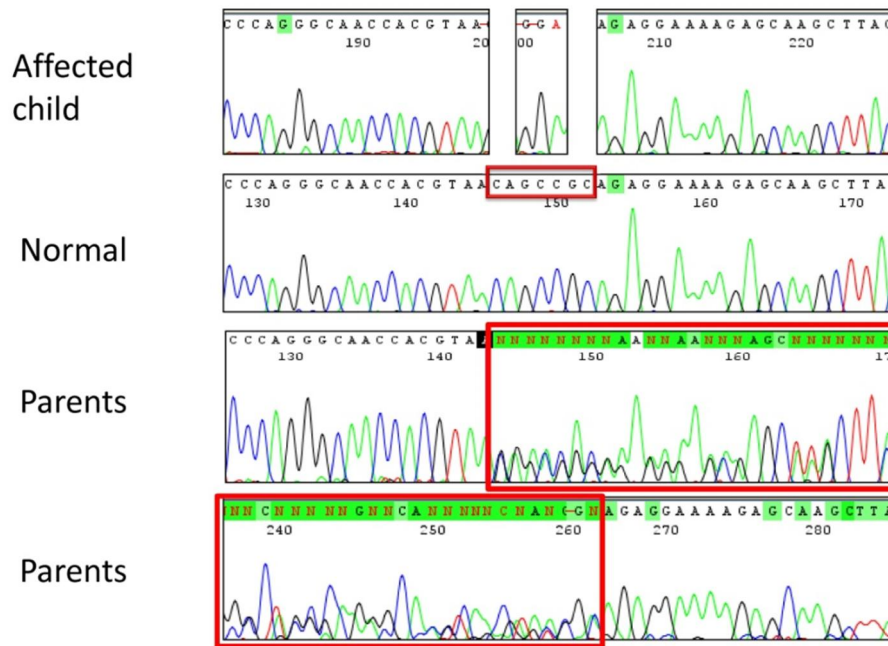
DNA strand. Primers are listed in the Table1.

We identified a truncating homozygote mutation in exon 2 of *BTD* [c.98\_104del7ins3 (p.Cys33PhefsX36)] in the affected child. Her parents were also heterozygote carrier for this mutation (Fig.1).

The [c.98\_104del7ins3 (p.Cys33PhefsX36)] mutation is a complex deletion/insertion mutation: deleting 7bp from position 98 to 104 and at the same time inserting 3bp, which leads to a frameshift, starting from residue 33 and ending to a stop codon in residue 36 of *BTD*.

**Table 1** primers used for sequencing of entire coding region of *BTD*

Primer Name	Sequence
BTD_1F	CCTGCCATCTGATAACAGAC
BTD_1R	CTGACTTAGATCACCTCTGTG
BTD_2F	gcaggattctttattcagctg
BTD_2R	gcaatctgctctgtatgagag
BTD_3F	cctgccatctgataacagac
BTD_3R	ctgacttagatcacctctgtg
BTD_4.1F	GGGTGGTCTCAATCTCCTGA
BTD_4.1R	GGGTGTGTATGCCACTTCCT
BTD_4.2F	GTGTACCCAACTGCCTGGAT
BTD_4.2R	AGGTGGCCTCTCGTAAAGT
BTD_4.3F	ATTTTGTGAGGCGATCCGTA
BTD_4.3R	AGCCCAGAGGACAGCCTACT



**Figure 1** sequence chromatographs for the surrounding region of the c.98\_104del7ins3 (p.Cys33PhefsX36) mutation in the affected child, a healthy control as well as one of the parents. The affected child is homozygously mutated but parent is heterozygote carrier. The sequence peaks of the parent are getting doubled exactly after starting of the heterozygous mutation borders from each side.

In view of the fact that BTDD is a treatable disorder, early detection and permanent management of patients with BTDD by newborn screening can be a cost-effective tool to prevent the rebellious seizure and all the neurological sequel of this disorder.

Biotinidase deficiency should be contemplated in children with seizures particularly in those with additional clinical expressions of this disease including oncoming neurological or (and) cutaneous manifestations.

If left untreated, this disorder can lead to neurological and cutaneous symptoms, such as severe illness, hearing loss, eye abnormalities and loss of vision, problems with movement and balance (ataxia), skin manifestation (dermatitis), hair loss (alopecia), seborrhea, psychomotor delay, conjunctivitis, irreversible neurological deterioration, encephalopathy, paraparesis, retardation, epilepsy exceptionally and a fungal infection called candidiasis, spasticity and also hypertonia (Wolf, Grier et al. 1983; Pindolia, Jordan et al. 2011; Wolf 2011).

With an early verification by newborn screening, immediate treatment and lifelong management with biotin supplements many of these complications can be prevented.

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