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RESEARCH NOTE

Identification of a novel *GLB1* mutation in a consanguineous Pakistani family affected by rare infantile GM1 gangliosidosis

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Abstract. Monosialotetrahexosylganglioside (GM1) is a rare lysosomal storage disorder caused by the deficiency of beta-galactosidase (β-Gal) encoded by galactose beta 1 (*GLB1*). It is clinically characterized by developmental delay attributed to multifold accumulation of GM1 gangliosides in nerve cells. In this study, we present a case of infantile GM1 gangliosidosis in a consanguineous Pakistani family. The child was presented with developmental delay, hepatosplenomegaly and recurrent chest infections at 7.5 months of age. Radiological and biochemical investigations including magnetic resonance imaging (MRI), bone marrow biopsy and urine oligosaccharide analyses suggested lysosomal storage disorder. Significantly low levels of β-Gal enzyme confirmed the diagnosis of GM1 gangliosidosis. DNA sequencing of *GLB1* identified a homozygous 2-bp deletion c.881-882delAT (p.Tyr294Terfs) in exon 8. *In silico* analysis supported the deleterious effect of the variant. This study extends *GLB1* mutation spectrum and should benefit genetic counselling and prenatal diagnosis of the affected family.

Keywords. GM1 gangliosidosis; beta-galactosidase; novel mutation; *GLB1* gene.

Introduction

GM1 gangliosidosis is a rare autosomal recessive lysosomal storage disorder with an estimated global incidence of 1:1,00,000–2,00,000. It is caused by the deficiency of hydrolytic β-Gal encoded by the *GLB1* gene located on chromosome 3p21.33 (Nishimoto *et al.* 1991; Yoshida *et al.* 1991). The disease manifestation is the outcome of elevated accumulation of GM1 gangliosides in different organs particularly the brain. Owing to the deficiency of the β-Gal enzyme, the patients are incapable of cleaving β-galactose from GM1 gangliosides and other substrates resulting in accumulation of GM1 gangliosides and additional substrates in different organs according to their primary sites of biosynthesis (Sandhoff and Harzer 2013).

GM1 gangliosidosis has been described as a severe neurological disease clinically classified into three distinct forms: type I (OMIM: 230500), type II (OMIM: 230600) and type III (OMIM: 230650). The activity of β-Gal is almost completely lost in severe infantile type I disease with <1% residual enzyme activity. The other two forms have weak residual activity of the enzyme thus establishing inverse correlation of enzyme activity with disease severity (Callahan 1999).

Mutations in the *GLB1* also cause Morquio-B syndrome for which the exact mechanism is yet to be elucidated, however, the distribution of *GLB1* mutations associated with Morquio-B syndrome has been observed to have a predisposition to the vicinity of ligand-binding pocket of β-Gal (Ohto *et al.* 2012). GM1 gangliosidosis and Morquio-B syndrome represent two extreme ends of heterogeneous continuum with differential neurological deterioration in GM1 gangliosidosis patients while relatively invariable neurological retainment in Morquio-B syndrome patients (Hofer *et al.* 2010).

The *GLB1* gene contains 16 exons spanning 62.5 kb region (Takano and Yamanouchi 1993). It transcribes into two alternatively spliced mRNAs: (i) a major

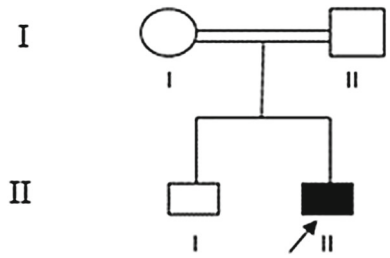

Figure 1. Pedigree of the family; arrow indicates the proband.

transcript of 2.5 kb encoding β-Gal translated into 677 amino acid residues with 23 amino acids acting as signal sequences targeting the enzyme to the lysosomal compartment (Oshima *et al.* 1988) and (ii) a shorter transcript of 2 kb encoding the elastin-binding protein (EBP) that is localized in cellular membrane and is involved in assembly of tropoelastin monomers into growing elastin fibres. β-Gal forms a multiprotein complex with protective protein cathepsin A (PPCA) and alpha neuraminidase (NEU1) and galactosamine 6-sulphate sulphatase (GALNS) inside lysosomes while EBP binds to PPCA and NEU1 on the cell surface (Hinek *et al.* 2000; Caciotti *et al.* 2005). To date, 146 mutations have been reported in *GLB1* in association with GM1 gangliosidosis (www.hgmd.cf.ac.uk). In this study, a novel *GLB1* mutation was identified in a Pakistani patient affected with GM1 gangliosidosis.

Materials and methods

Human subjects

Peripheral blood samples were collected from all family members after obtaining informed consent. The proband II-II (figure 1) was clinically evaluated by the Department of Pediatric Gastroenterology, The Children's Hospital and The Institute of Child Health, Lahore and referred for biochemical and molecular diagnosis. This study was approved by the institutional review board of Quaid-i-Azam University, Islamabad, Pakistan.

Biochemical and mutation analyses

After confirmation of the clinical suspicion through enzyme analysis (β-Gal assay), the patient's sample was subjected to molecular screening for the *GLB1* gene. The genomic sequence was derived from the GenBank (NG_009005.1) and nucleotide numbering was based on the cDNA reference sequence (NM_000404.3) as per the Human Genome Variation Society (HGVS) nomenclature with A of the start codon ATG as +1. All 16 exons and intron–exon boundaries of *GLB1* were polymerase chain reaction (PCR) amplified in thermocycler (Labnet International) and the PCR products were purified using GeneJET PCR Purification kit (Thermo Fisher Scientific). The primers were designed manually from flanking sequences and are available upon request. The purified PCR products were subjected to the Sanger sequencing reaction using BigDye Terminator v3.1 Cycle Sequencing kit (Thermo Fisher Scientific). The sequencing reaction products were purified by the ethanol precipitation method and run on an ABI Prism 3730 genetic analyzer. The sequences were analyzed using BioEdit sequence alignment editor ver. 7.0.5.3 (Hall 1999).

Results

The proband (figure 1, II-II) was from Swabi city of Khyber Pakhtunkhwa province of Pakistan and was clinically evaluated by the Children's Hospital and The Institute of Child Health, Lahore at the age 7.5 months for the evaluation of recurrent chest infections, developmental delay and hepatosplenomegaly. His parents were first cousins and are Pathan by cast. The elder brother of proband is healthy with no disease history. At the time of presentation, the proband's haemoglobin level was 10.3 g/dL, WBC count was 7.5×10^3/mm^3 and platelet count was 201×10^6/mm^3. Liver and kidney function tests were normal. Eye examination showed bilateral cherry-red spots. Microscopic examination of bone marrow biopsy showed lipid-laden macrophages and the brain MRI showed prominent ventricular and extraventricular spaces. Sphingomyelinase enzyme activity was normal which ruled out Niemann Pick disease. The urine was subjected to thin layer chromatography for oligosaccharides and found positive for GM1 gangliosidosis. β-Gal assay showed remarkably deficient enzyme activity (0.87 nmol/h/mg protein, reference range: 32.5–206.5 nmol/h/mg protein). During the follow-up period, the proband showed a relentless neurodegeneration with failure to thrive, hypotonia and recurrent chest infections, required one time hospitalization and multiple courses of oral antibiotics were taken at home. At 13 months of age, the proband succumbed to severe malnutrition and respiratory failure.

The Sanger sequencing analysis of the *GLB1* gene of the proband (II-II) indicated that he was homozygous for a novel frameshift variant c.881-882delAT (rs767704163) in exon 8 (table 1) (figure 2a) with a minor allele frequency of 0.00001657 according to the Exome Aggregation Consortium (EXAC) database (exac.broadinstitute.org). *In silico* tools MutationTaster and PROVEAN predicted the variant as disease causing and deleterious, respectively. Family members were subjected to sequencing of exon 8 for segregation analysis which showed that the parents were heterozygous for the mutation while the healthy brother was

Table 1. Summary of variants identified in this study.

SNP ID	cDNA position	Protein change	*In silico* prediction	MAF	References
rs7637099	c.29C>T	p.Pro10Leu	Benign	0.43[a] 0.56[b]	Gururaj *et al.* (2005)
rs4302331	c.1561T>C	p.Cys521Arg	Benign* Low penetrant-disease causing[#]	0.07[a] 0.98[b]	Santamaria *et al.* (2007)
rs767704163	**c.881-882delAT**	**p.Tyr294Terfs**	**Pathogenic***	**0.00001657**[b]	**Novel**

*As per American College of Medical Genetics (ACMG) guidelines (Richards *et al.* 2015).
[#]Santamaria *et al.* (2007) classified the allele as a low penetrant-disease causing allele with 25% of residual enzyme activity of otherwise 'benign' significance.
[a]MAF from the ClinVar database (www.ncbi.nlm.nih.gov/clinvar).
[b]MAF from the EXAC database (http://exac.broadinstitute.org).
The novel causative variant identified in the current study are in bold.

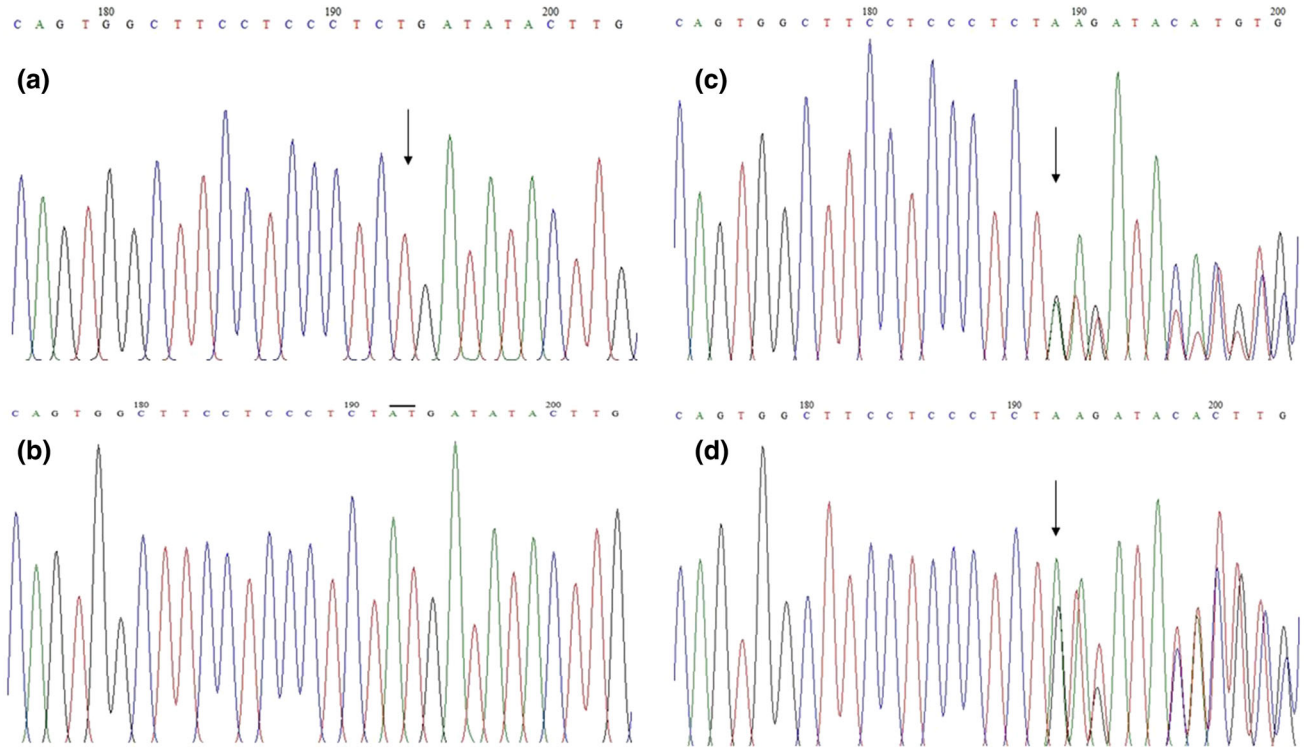

Figure 2. Sequencing chromatograms of GLB1 exon 8 showing (a) homozygous 2-bp deletion (c.881-882delAT) in proband II-II, (b) homozygous wild-type sequence in the healthy sibling II-I, (c) heterozygous 2-bp deletion in the mother (I-I) and (d) heterozygous 2 bp deletion in the father (I-II). Arrows indicate the position of the deletion and the bar above the normal sequence (b) indicates the deleted AT nucleotides.

found homozygous for the wild-type allele (figure 2, b–d). In addition to the frameshift variant, two missense variants were identified in the proband: c.29C>T (p.Pro10Leu) (rs7637099) in exon 1 and c.1561T>C (p.Cys521Arg) (rs4302331) in exon 15. These were considered to be neutral polymorphisms based on their minor allele frequencies (0.56 for c.29C>T and 0.98 for c.1561T>C) (table 1).

Discussion

Human β-Gal is an enzyme encoded by the *GLB1* gene that removes beta-ketosidically linked galactose residues from glycoproteins, sphingolipids and keratin sulphates within the lysosomes. The enzyme consists of three domains. The first is the distinct catalytic triosephosphate isomerase (TIM)-barrel domain (1–359) while the

second (397–514) and third (545–647) domains comprise of galactose-binding domain-like folds named as beta domain 1 and beta domain 2. The TIM-barrel domain links to the beta-domain 1 through TIM-beta 1 loop (360–396) (Ohto *et al.* 2012).

In this study, a patient with an infantile GM1 gangliosidosis phenotype has been identified with a novel deletion mutation c.881-882delAT (p.Tyr294Terfs) in the catalytic domain of β-Gal (TIM-barrel domain). This deletion mutation predicts a stop codon at 294th residue of the otherwise 677 amino acid long polypeptide. Therefore, the resultant truncated protein lacks major portion of the β-Gal enzyme and EBP and is likely associated with complete deficit of enzyme activity. The mutation is in exon 8 which is common to both transcripts of the *GLB1* gene, thus might affect the elastogenesis process as well (Santamaria *et al.* 2006), but the patient was not evaluated for it.

The phenotypic manifestations of the proband agree with studies conducted previously demonstrating the adverse functional effects of the premature truncation of the *GLB1* gene (Santamaria *et al.* 2006; Hofer *et al.* 2010; Caciotti *et al.* 2011). In evidence of the diminished levels of enzyme activity in peripheral blood leucocytes, a correlation has been sought through *in silico* evaluation of the allele through MutationTaster and PROVEAN as 'deleterious' and 'disease causing', respectively.

β-Gal enzyme activity measured in peripheral blood leucocytes of the patient in this study was significantly low. However, expression study of the frameshift allele in cultured fibroblasts is suggested to further confirm the diminished enzyme activity. In the previous studies, no enzyme activity could be observed with prematurely truncating mutations in cultured cells (Hinek *et al.* 2000; Hofer *et al.* 2010; Caciotti *et al.* 2011) except the artificially introduced mutation p.K659X (19 amino acids before the natural stop codon) in exon 16 that had normal catalytic activity (Hofer *et al.* 2010).

The identified missense variants c.29C>T (p.Pro10Leu) and c.1561T>C (p.Cys521Arg) were reported to be SNPs with allele frequencies of 0.56 and 0.98, respectively, with 'benign' significance according to the EXAC database. Both variants have been previously described as silent polymorphisms (c.29C>T) and mild phenotype alleles (c.1561T>C) (Gururaj *et al.* 2005; Santamaria *et al.* 2007).

In conclusion, a novel *GLB1* mutation causing GM1 gangliosidosis is identified in this study. This study expands the spectrum of *GLB1* mutations and is helpful in prenatal diagnosis and genetic counselling of the affected family.

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