Research note

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

Bibi Zubaida (MPhil)[1], Muhammad Almas Hashmi (FCPS)[2], Huma Arshad Cheema (MRCP)[2], Muhammad Naeem (PhD)[1]*

1. Medical Genetics Research Laboratory, Department of Biotechnology, Quaid-i-Azam University, Islamabad, Pakistan

2. Department of Pediatric Gastroenterology, The Children's Hospital and the Institute of Child Health, Lahore, Pakistan

*Corresponding author

Short Title: Novel *GLB1* mutation in GM1 gangliosidosis

Source of support: This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors. B.Z. is supported by Higher Education Commission of Pakistan through Indigenous Ph.D. Fellowship (PIN: 213-52789-2BM2).

Conflict of interest: The authors declare no conflict of interest.

Address for correspondence: Muhammad Naeem, Medical Genetics Research Laboratory, Department of Biotechnology, Quaid-i-Azam University, Islamabad, Pakistan. Email: mnaeemqau@gmail.com

Keywords

GM1 gangliosidosis, beta-galactosidase, novel mutation, *GLB1*

Introduction

GM1 gangliosidosis is a rare lysosomal storage disorder caused by the deficiency of beta-galactosidase encoded by *GLB1* (Galactose Beta 1). It is clinically characterized by developmental delay attributed to multifold accumulation of GM1 gangliosides in nerve cells. In the current 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 MRI (Magnetic Resonance Imaging), bone marrow biopsy and urine oligosaccharides analysis suggested lysosomal storage disorder. Significantly low levels of beta-galactosidase 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. The study extends *GLB1* mutation spectrum and should benefit genetic counselling and prenatal diagnosis of the affected family.

GM1 (monosialotetrahexosylganglioside) 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 beta-galactosidase (β-Gal) encoded by *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 brain. Owing to the deficiency of β-Gal enzyme, the patients are incapable to cleave 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 as 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 heterogenous continuum with differential neurological deterioration in GM1 gangliosidosis patients while relatively invariable neurological retainment in Morquio-B syndrome (Hofer *et al*. 2010).

GLB1 gene contains 16 exons spanning 62.5Kb region (Takano and Yamanouchi 1993). It transcribes into two alternatively spliced mRNAs: (a) a major transcript of 2.5Kb encoding β-Gal translated into 677 amino acid residues with 23 amino acids acting as signal sequence targeting the enzyme to lysosomal compartment (Oshima *et al*. 1988) and (b) a shorter transcript of 2Kb encoding the elastin-binding protein (EBP) that is localized in cellular membrane and is involved in assembly of tropoelastin monomers into growing elastin fibers. 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 the

present study, a novel *GLB1* mutation was identified in a Pakistani patient affected with GM1-gangliosidosis.

Material and Methods

Human subjects

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

Biochemical and mutation analysis

After confirmation of the clinical suspicion through enzyme analysis (β-Gal assay), the patient's sample was subjected to molecular screening for *GLB1* gene. The genomic sequence was derived from Genbank (NG_009005.1) and nucleotide numbering was based on cDNA reference sequence (NM_000404.3) as per HGVS (Human Genome Variation Society) 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 Sanger sequencing reaction using BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific). The sequencing reaction products were purified by ethanol precipitation method and run on an ABI Prism 3730 genetic analyzer. The sequences were analyzed using BioEdit sequence alignment editor version 7.0.5.3 (Hall 1999).

Results

The child (Fig1; II-II) at 7.5 months of age was referred from Swabi city of Khyber Pakhtunkhwa province of Pakistan for evaluation of recurrent chest infections, developmental delay and hepatosplenomegaly. His parents were first cousins and Pathan by cast. He had one elder male sibling who was alive and healthy with no disease history. At the time of presentation, his hemoglobin was 10.3 g/dL, WBCs 7.5 x 10^3/mm^3 and platelets 201 x 10^6/mm^3. Liver and kidney function tests were unremarkable. Eye examination showed bilateral cherry red spots. Microscopic examination of bone marrow biopsy showed lipid laden macrophages and brain MRI showed prominent ventricular and extraventricular spaces. Sphingomyelinase enzyme activity was normal that 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/hour/mg protein (reference range: 32.5-206.5 nmol/hour/mg protein)]. During follow up period, the child showed a relentless neurodegeneration with failure to thrive, hypotonia and recurrent chest infections requiring one hospital admission and multiple courses of oral antibiotics at home. At 13 months of age he succumbed to severe malnutrition and respiratory failure.

The Sanger sequencing analysis of *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) (Fig 2-A) with minor allele frequency of 0.00001657 according to EXAC (Exome Aggregation Consortium) 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 parents were heterozygous for the mutation while healthy brother was found homozygous for wild type allele

(Fig2-B,C,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 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 *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 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 beta-domain 1 via TIM-beta1 loop (360-396) (Ohto *et al*. 2012).

In the current study, a patient with 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 otherwise 677 amino acid long polypeptide. Therefore, the resultant truncated protein lacks major portion of β-Gal enzyme and EBP and is likely associated with complete deficit of enzyme activity. The mutation is in exon 8 that is common to both transcripts of *GLB1* gene thus might affect the elastogenesis process as well (Santamaria *et al*. 2006), but the patient had not been evaluated for it.

The phenotypic manifestations of the proband agree with studies conducted previously demonstrating the adverse functional effects of premature truncation of *GLB1* gene (Santamaria *et al*. 2006; Hofer *et al*. 2010; Caciotti *et al*. 2011). In evidence of diminished levels of enzyme

activity in peripheral blood leukocytes, 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 leukocytes of the patient in the current 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 one artificially introduced mutation p.K659X (19 amino acids before 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 SNPs with allele frequencies of 0.56 and 0.98, respectively with 'benign' significance according to EXome Aggregation Consortium (EXAC) database [exac.broadinstitute.org]. Both variants have been previously described as silent polymorphism (c.29C>T) and mild phenotype allele (c.1561T>C) (Gururaj *et al*. 2005; Santamaria *et al*. 2007).

In conclusion, a novel *GLB1* mutation causing GM1 Gangliosidosis is identified in the current study. This study expands the spectrum of *GLB1* mutations and should be helpful in prenatal diagnosis and genetic counseling of the affected family.

Acknowledgements

We are thankful to the family members for participation in the study. Higher Education Commission of Pakistan is acknowledged for supporting B.Z. under Indigenous Ph.D. Fellowship Scheme (PIN: 213-52789-2BM2).

Funding

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

References

Caciotti A., Donati M. A., Boneh A., d'Azzo A., Federico A., Parini R. *et al*. 2005 Role of beta-galactosidase and elastin binding protein in lysosomal and nonlysosomal complexes of patients with GM1-gangliosidosis. *Hum Mutat*. **25**, 285-292.

Caciotti A., Garman S. C., Rivera-Colon Y., Procopio E., Catarzi S., Ferri L. *et al*. 2011 GM1 gangliosidosis and Morquio B disease: an update on genetic alterations and clinical findings. *Biochim Biophys Acta*. **1812**, 782-790.

Callahan J. W. 1999 Molecular basis of GM1 gangliosidosis and Morquio disease, type B. Structure-function studies of lysosomal beta-galactosidase and the non-lysosomal beta-galactosidase-like protein. *Biochim Biophys Acta*. **1455**, 85-103.

Gururaj A., Sztriha L., Hertecant J., Johansen J. G., Georgiou T., Campos Y. *et al*. 2005 Magnetic resonance imaging findings and novel mutations in GM1 gangliosidosis. *J Child Neurol*. **20**, 57-60.

Hall T. A. 1999 BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acids Symp Ser*. **41,** 95–98.

Hinek A., Zhang S., Smith A. C. and Callahan J. W. 2000 Impaired elastic-fiber assembly by

fibroblasts from patients with either Morquio B disease or infantile GM1-gangliosidosis is linked to deficiency in the 67-kD spliced variant of beta-galactosidase. *Am J Hum Genet*. **67**, 23-36.

Hofer D., Paul K., Fantur K., Beck M., Roubergue A., Vellodi A. *et al*. 2010 Phenotype determining alleles in GM1 gangliosidosis patients bearing novel GLB1 mutations. *Clin Genet*. **78**, 236-246.

Nishimoto J., Nanba E., Inui K., Okada S. and Suzuki K. 1991 GM1-gangliosidosis (genetic beta-galactosidase deficiency): identification of four mutations in different clinical phenotypes among Japanese patients. *Am J Hum Genet*. **49**, 566-574.

Ohto U., Usui K., Ochi T., Yuki K., Satow Y. and Shimizu T. 2012 Crystal structure of human beta-galactosidase: structural basis of Gm1 gangliosidosis and morquio B diseases. *J Biol Chem*. **287**, 1801-1812.

Oshima A., Tsuji A., Nagao Y., Sakuraba H. and Suzuki Y. 1988 Cloning, sequencing, and expression of cDNA for human beta-galactosidase. *Biochem Biophys Res Commun*. **157**, 238-244.

Richards S., Aziz N., Bale S., Bick D., Das S., Gastier-Foster J. *et al*. 2015 Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. **17**, 405-424.

Sandhoff K. and Harzer K. 2013 Gangliosides and gangliosidoses: principles of molecular and metabolic pathogenesis. *J Neurosci*. **33**, 10195-10208.

Santamaria R., Chabas A., Callahan J. W., Grinberg D. and Vilageliu L. 2007 Expression and characterization of 14 GLB1 mutant alleles found in GM1-gangliosidosis and Morquio B patients. *J Lipid Res*. **48**, 2275-2282.

Santamaria R., Chabas A., Coll M. J., Miranda C. S., Vilageliu L. and Grinberg D. 2006 Twenty-one novel mutations in the GLB1 gene identified in a large group of GM1-gangliosidosis and Morquio B patients: possible common origin for the prevalent p.R59H mutation among gypsies. *Hum Mutat*. **27**, 1060.

Takano T. and Yamanouchi Y. 1993 Assignment of human beta-galactosidase-A gene to 3p21.33 by fluorescence in situ hybridization. *Hum Genet*. **92**, 403-404.

Yoshida K., Oshima A., Shimmoto M., Fukuhara Y., Sakuraba H., Yanagisawa N. *et al*. 1991 Human beta-galactosidase gene mutations in GM1-gangliosidosis: a common mutation among Japanese adult/chronic cases. *Am J Hum Genet*. **49**, 435-442.

Received 2 March 2018; revised 2 April 2018; 5 April 2018

Table 1: Summary of variants identified in the current study

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

*As per ACMG (American College of Medical Genetics) guidelines (Richards *et al*. 2015).

#Santamaria, *et al*. classified the allele as low penetrant disease causing allele with 25% of residual enzyme activity of otherwise 'Benign' significance.

[a] MAF from ClinVar database (www.ncbi.nlm.nih.gov/clinvar)

[b] MAF from EXAC database (http://exac.broadinstitute.org).

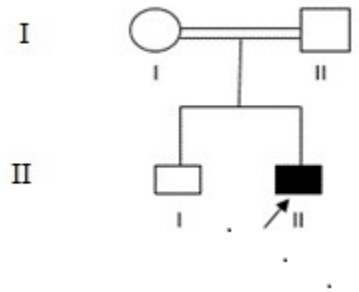

Figure 1: Pedigree of the family; arrow indicates the proband.

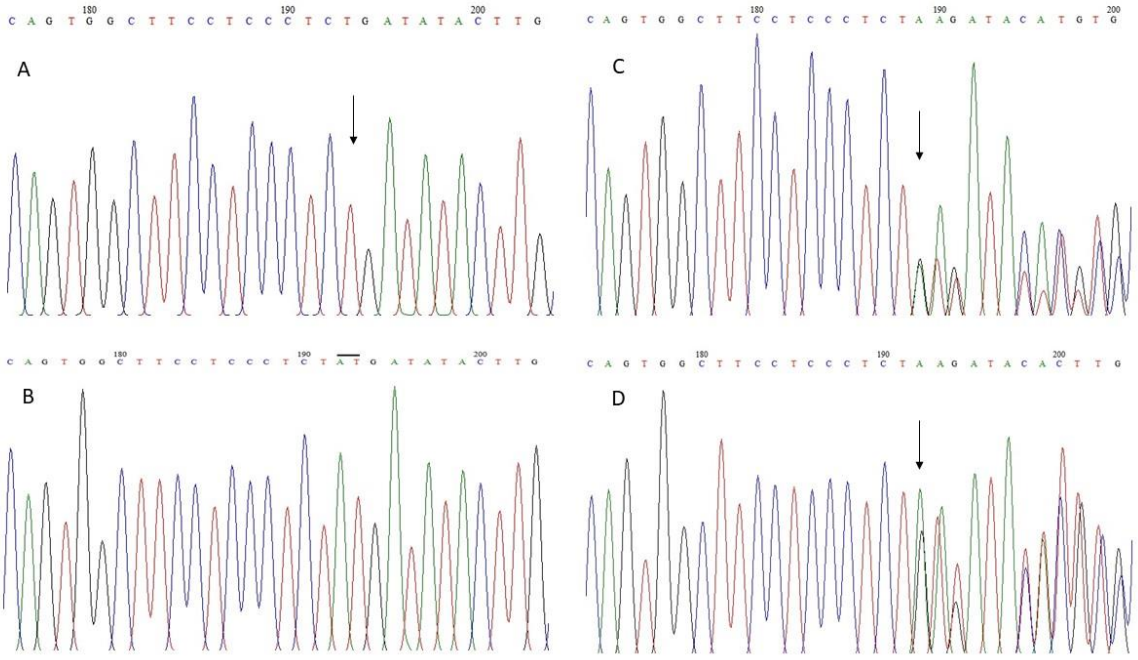

Figure 2: Sequencing chromatograms of *GLB1* exon 8 showing (A) homozygous 2-bp deletion (c.881-882delAT) in the proband II-II, (B) homozygous wild type sequence in the healthy sibling II-I, (C) heterozygous 2-bp deletion in the mother (I-I) and (D) heterozygous 2bp deletion in the

father (I-II). Arrows indicate position of the deletion and the bar above the normal sequence (B) indicates the deleted AT nucleotides.