

Identification of a Novel *TAZ* Gene Mutation in a Family With X-Linked Dilated Cardiomyopathy Barth Syndrome

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Abstract

Mutations in the tafazzin (*TAZ*) gene on chromosome Xq28 are responsible for the Barth syndrome (BTHS) phenotype resulting in a loss of function in the protein tafazzin involved in the transacylation of cardiolipin, an essential mitochondrial phospholipid. *TAZ* gene was investigated in the proband in our study, who died of dilated cardiomyopathy at 8 months of age, and his family by sequencing to identify the genetic cause of BTHS. Molecular analysis revealed a novel mutation in exon 5 (c.520T>G) of the *TAZ* gene. This novel mutation c.520T>G, p.W174G, was also found in female carriers (mother and grandmother of proband) in the family. Bioinformatic analysis was carried out to examine the effect of mutation in the gene and confirmed the deleterious effect of this single mutation to the protein structure. Protein modeling and 3-dimensional structure of *TAZ* protein demonstrated the significantly visible changes in mutated protein leading to BTHS phenotype. Prenatal diagnosis in a subsequent pregnancy showed a carrier female, and pregnancy was continued. Child is doing well at 1 year of age.

Keywords

Barth syndrome, *TAZ* gene, India, prenatal diagnosis, bioinformatic analyses

Introduction

Barth syndrome (BTHS; Online Mendelian Inheritance in Man [OMIM] accession no. 302060) is an X-linked disorder caused by a mutation in *TAZ* (G4.5 or TAFAZZIN) gene.¹ *TAZ* is involved in the metabolism of the mitochondrial-specific phospholipid cardiolipin (CL),^{2,3} loss of which results in skeletal and cardiac myopathy and cyclic neutropenia. Heart failure is the main cause of death in infancy, followed by sepsis due to neutropenia, which causes some variability in the clinical course of BTHS.⁴ Mutations in the *TAZ* gene cause tafazzin deficiency, and sequence analysis of this gene is necessary to confirm the diagnosis of BTHS and corroborate with clinical and biochemical findings. The human *TAZ* gene contains 11 short exons and 10 variably long introns. Human Gene Mutation Database Professional⁵ reports a total of 120 *TAZ* gene mutations of which 35.6% are missense, 12.5% are nonsense, 18.3% affect splicing, 19.2% are microdeletions, 7.7% are microinsertions, and 6.7% are large gene rearrangements.^{6,7} Frameshift mutations causing tafazzin truncation and mutations affecting splice donor or acceptor sites have also been

identified.⁸ In the present study, we made a postmortem diagnosis of BTHS in an infant who died of dilated cardiomyopathy with left ventricular (LV) noncompaction, based on family history and characteristic findings, and analyzed *TAZ* gene in the proband and his family.

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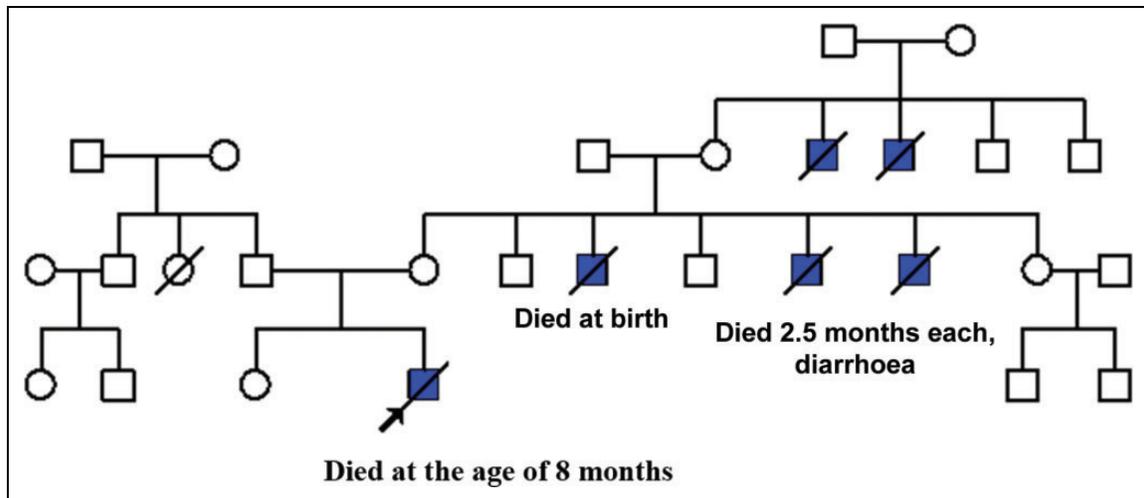


Figure 1. Pedigree of the studied family.

Patient

Case History

MG was born at term, with birth weight of 2.5 kg, and was discharged with mother after an uneventful perinatal period. He was hospitalized on day 11 because of respiratory difficulty and treated for pneumonia. The echocardiogram had shown left ventricular ejection fraction (LVEF) of 45%. The LVEF reduced to 15% at 5 months, when he had an acute episode of respiratory distress. A dilated cardiomyopathy and absolute neutropenia (absolute neutrophil count of 303) were noted on sepsis screen (white blood cell 4.21×10^3 , neutrophils 7.2%, lymphocytes 64.6%, eosinophils 6.9%, and monocytes 21.1%). There was also history of persistent diarrhea. He remained unwell with floppiness, weak suck, and persistent diarrhea in subsequent months. He did not respond to change in formula feeds. At 8 months, his echocardiogram showed a dilated cardiomyopathy of noncompaction type, severe LV dysfunction (LVEF = 10%), and dilated left atrium and left ventricle—all suggestive of severe congestive heart failure with peripheral circulatory collapse. He died a few days after acute deterioration at 8 months of age. Tandem mass spectrometry for acylcarnitine profile and enzyme assay for Pompe disease were performed in the perimortem period, both of which were normal. DNA was stored. After death, a detailed family history was taken and note made of infant deaths due to diarrhea in 3 maternal uncles and 2 mother's maternal uncles (Figure 1). In view of the clinical presentation with dilated and LV noncompaction type of cardiomyopathy, and neutropenia on a background family history highly suggestive of an X-linked disorder, a clinical diagnosis of BTHS was made and gene studies performed.

Molecular Analyses

The exons with exon–intron boundaries of *TAZ* gene were polymerase chain reaction (PCR) amplified (Table 1) including

Table 1. Oligonucleotide Primers Used in PCR Amplifications and Sequencing.

Seq ID	Primer Seq
TAZ 1-2 F	5' GTCAGGGGCCAGTGTCTC 3'
TAZ 1-2 R	5' CGAATCCTCGCCTTTCT 3'
TAZ 3-4 F	5' CCCTGGGGATATGGGAAG
TAZ 3-4 R	5' TTTCTGCCCCAGAAGTG
TAZ 5-6 F	5' GTAAGCTAACCTGTCACCCAC
TAZ 5-6 R	5' CAGAGACCAGCAGCCCTTAC
TAZ 7-8 F	5' GCACAGAAGCTTGGCTCAG
TAZ 7-8 R	5' GAGACACGGAGCAGACAGC
TAZ 9-10 F	5' GGCTGTGGCACTCCTACT
TAZ 9-10 R	5' CCTCAGCCCCAGCTCTGT

Abbreviations: f, forward; PCR, polymerase chain reaction; R, reverse; seq, sequence.

regions believed to be hot spots for gene mutations in DNA samples of the proband and his family. The PCR products were directly sequenced (Big Dye Terminator Cycle Sequencing Kit and ABI Prism 310; Perkin Elmer Applied Biosystems, Norwalk) for mutation detection using the same primers as for PCR. Obtained sequences were compared to the *TAZ* gene sequence (NCBI GenBank Accession Numbers X92763 and X92764).

Bioinformatic Analyses

Bioinformatic analysis was carried out to determine the functional consequences of mutation and its effect on protein structure using different tools, for example, Polyphen2 (genetics.bwh.harvard.edu/pph2/), Mutationtaster (www.mutationtaster.org/), Sorting Intolerant From Tolerant (SIFT; sift.jcvi.org), Support Vector Machine and Neural (<http://www.springerlink.com/content/k238jx04hm87j80>), and 3-dimensional protein modeling structure for mutated protein (www.expasy.org/proteomics/protein_structure).

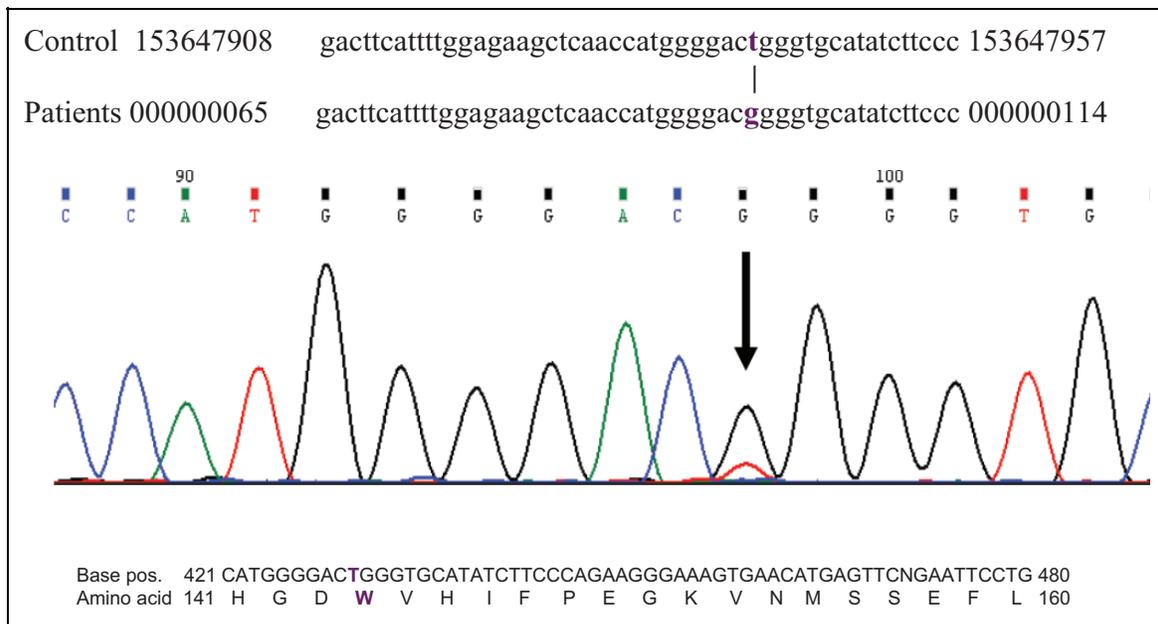


Figure 2. Electropherogram showing the DNA sequence of the patient for exon 5 in TAZ gene.

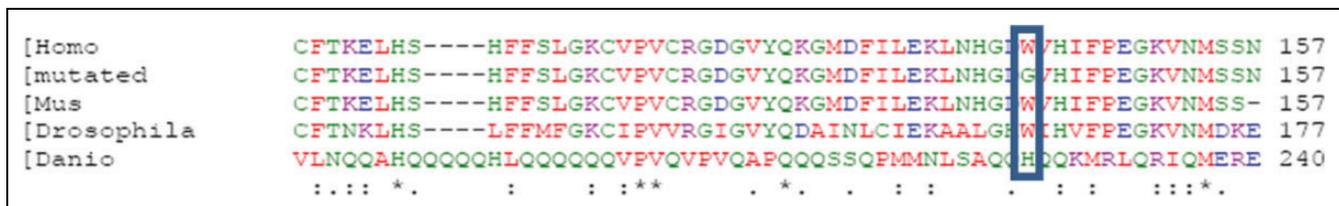


Figure 3. Partial alignment of TAZ gene to show conservation of the residues found to be mutated in human TAZ.

Result

Mutation analysis for the Tafazzin or TAZ gene (OMIM 300394, TAZ) in our proband revealed a novel thymine to guanine transversion mutation at position c.520 in exon 5, resulting in a predicted p.Try174Gly amino acid substitution (Figure 2). Family studies showed that the proband's mother, maternal aunt, and grandmother carried the same mutation (Figure 1). Subsequent bioinformatics analyses showed high prediction of the sequence alteration to be deleterious based on PolyPhen (0.999), SIFT (0), and MutationTaster (0.999999). Support Vector Machine and Neural Network had demonstrated the effect of mutation as a decrease in the stability of protein structure with efficient predictive confidence scores: -0.63077206 and -0.64898906859 , respectively. The protein prediction software demonstrated mutant residue to be smaller than the wild-type residue. The mutation may cause an empty space and loss of hydrophobic interactions in the core of the protein. The mutant residue is located near a highly conserved position (Figure 3). The residue is buried in the core of a domain and mutant residue might disturb the core structure of this domain. ERRAT server predicted secondary structure for both the proteins that show mutation site forming coil structure (Figure 4). Because of differences in binding of tryptophan (Trp) and glycine (Gly),

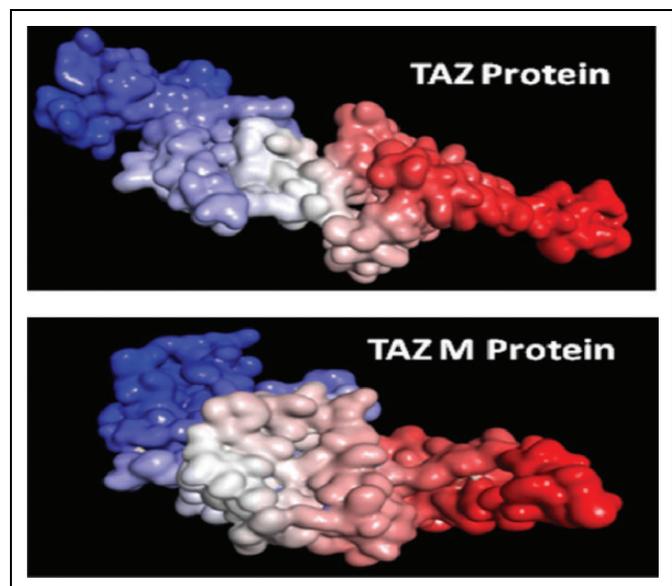


Figure 4. TAZ M having more complex structure than TAZ protein.

the total protein structure changed in TAZ mutated (TAZ M) protein (Figure 5), probably damaging the protein.

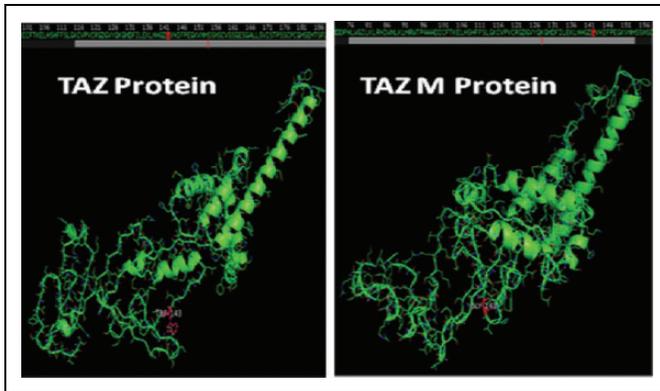


Figure 5. Position of Trp143 and Gly143 in the structure of taffazin (TAZ) from *Homo sapiens*.

Prenatal Diagnosis

Prenatal diagnosis was carried out in a subsequent pregnancy in the family, and the fetus was noted to be female and a carrier for this novel mutation. The pregnancy was continued, and a healthy female baby was born who is doing well at 1 year of age.

Discussion

Barth syndrome is caused by mutations in *TAZ* gene, which lead to altered taffazin protein involved in the metabolism of the mitochondrial-specific phospholipid CL, a dimeric phosphoglycerolipid present predominantly in mitochondrial membranes.^{9,10} Recently, CL has been shown to be critical for the correct organization of ATP synthase assembly.¹¹ Mutations have been reported in all exons of *TAZ*, including a variant of unknown significance in exon 5.¹²⁻¹⁶ We found a novel mutation in exon 5, c.520T>G, which is so far unreported, causing an amino acid change from tryptophan to glycine. The wild-type and mutant proteins differ in size and truncated protein causes the empty space in core of the protein. The mutation introduces a glycine at this position, which is very flexible and can disturb the required rigidity of the protein at this position. Mutation of a 100% conserved residue is usually damaging for the protein. The mutant and wild-type residues are not very similar. Based on this conservation information, this mutation is probably damaging to the protein. The mutant residue is located near a highly conserved position. The hydrophobicity of the wild type and mutant residues differs. The mutation may cause loss of hydrophobic interactions in the core of the protein. The tryptophan in TAZ shows less bonding and is highly stable, thus showing less compact structure, while in TAZ M, glycine has higher bonding and less stability, showing mutated protein structure as more compact and less active.

Family study has shown the heterozygous mutation in female carriers in a consistently X-linked recessive pattern. The case history has revealed infant deaths due to diarrhea in 3 maternal uncles and 2 mother's maternal uncles. Historically, most boys with BTHS died during fetal life through to infancy from either heart failure or overwhelming infection.¹⁶ An

article published in 2005 showed that 70% of retrospectively diagnosed brothers of patients with BTHS had died before the diagnosis was established in the family.¹⁷ This contrasted with patients identified prospectively and managed proactively, for whom mortality had fallen to just 10%¹⁷ emphasizing the importance of early diagnosis. Molecular analysis enabled a successful prenatal diagnosis and confirmed the female child to be a carrier of this novel mutation, enabling a decision to continue pregnancy.

Authors' Note

Minal Borkar at the time of study was affiliated with Center of Medical Genetics, Sir Ganga Ram Hospital, New Delhi, India. This article does not contain any studies with human or animal subjects performed by any of the authors. Minal Borkar contributed to analysis and interpretation of data, haplotype analysis, and drafting the article (molecular methodology). Sunita Bijarnia-Mahay contributed to conception and design, clinical management of case and facilitating prenatal diagnosis, and drafting the article. Sudha Kohli contributed to analysis and interpretation of data, prenatal diagnosis, and drafting the article (molecular methodology). Monica Juneja contributed to clinical management of case prior to diagnosis and drafting the article (provided patient information). Yogesh Srivastava contributed to analysis and interpretation of data (computational analysis and bioinformatics) and drafting the article (methodology and results of bioinformatics). Renu Saxena contributed to analysis and interpretation of data and revising the article critically for important intellectual content. Ishwar C. Verma contributed to critical evaluation for important intellectual content.

Declaration of Conflicting Interests

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References

1. D'Adamo P, Fassone L, Gedeon A, et al. The X-linked gene *G4.5* is responsible for different infantile dilated cardiomyopathies. *Am J Hum Genet.* 1997;61(4):862-867.
2. Barth PG, Scholte HR, Berden JA, et al. X-linked mitochondrial disease affecting cardiac muscle, skeletal muscle and neutrophil leucocytes. *J Neurol Sci.* 1983;62:327-355.
3. Schlame M, Ren M. Barth syndrome, a human disorder of cardiolipin metabolism. *FEBS Lett.* 2006;580(23):5450-5455.
4. Ronvelia D, Greenwood J, Platt J, Hakim S, Zaragoza MV. Intrafamilial variability for novel *TAZ* gene mutation: Barth syndrome with dilated cardiomyopathy and heart failure in an infant and left ventricular noncompaction in his great-uncle. *Mol Genet Metab.* 2012;3(3):428-432.
5. Human Gene Mutation Database Professional v.2013.2; 2013. Web site. <https://portal.biobase-international.com>.
6. Chen R, Tsuji T, Ichida F, et al. Mutation analysis of the *G4.5* gene in patients with isolated left ventricular noncompaction. *Mol Genet Metab.* 2002;77(4):319-325.

7. Gonzalez IL. Barth syndrome: TAZ gene mutations, mRNAs, and evolution. *Am J Med Genet A*. 2005;134(4):409-414.
8. Johnston J, Kelley RI, Feigenbaum A, et al. Mutation characterization and genotype–phenotype correlation in Barth syndrome. *Am J Hum Genet*. 1997;61(5):1053-1058.
9. Gonzalvez F, D’Aurelio M, Boutant M, et al. Barth syndrome: cellular compensation of mitochondrial dysfunction and apoptosis inhibition due to changes in cardiolipin remodeling linked to tafazzin (TAZ) gene mutation. *Biochem Biophys Acta*. 2013;1832(8):1194-1206.
10. Lu B, Kelher MR, Lee DP, et al. Complex expression pattern of the Barth syndrome gene product tafazzin in human cell lines and murine tissues. *Biochem Cell Biol*. 2004;82(5):569-576.
11. Ferri L, Donati MA, Funghini S, et al. New clinical and molecular insights on Barth syndrome. *Orphanet J Rare Dis*. 1997;8(1):27.
12. Bione S, D’Adamo E, Maestrini AK, et al. A novel X-linked gene, G4.5 is responsible for Barth syndrome. *Nat Genet*. 1996;12(4):385-389.
13. Lakdawala NK, Funke BH, Baxter S, et al. Genetic testing for dilated cardiomyopathy in clinical practice. *J Card Fail*. 2012;18(4):296-303.
14. Rigaud C, Lebre AS, Touraine R, et al. Natural history of Barth syndrome: a national cohort study of 22 patients. *Orphanet J Rare Dis*. 2013;8:70.
15. Sakamoto O, Ohura T, Katsushima Y, et al. A novel intronic mutation of the *TAZ (G4.5)* gene in a patient with Barth syndrome: creation of a 5' splice donor site with variant GC consensus and elongation of the upstream exon. *Hum Genet*. 2001;109(5):559-563.
16. Sarah LN Clarke, Ann Bowron, Iris L Gonzalez, et al. Barth syndrome. *Orphanet J Rare Dis*. 2013;8:23.
17. Huhta JC, Pomerance HH, Barness EG. Clinicopathologic conference: Barth syndrome. *Fetal Pediatr Pathol*. 2005;24(4-5):239-254.