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, pW174G, 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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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 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).
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), the total protein structure changed in TAZ mutated (TAZ M) protein (Figure 5), probably damaging the protein.

**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.

**Figure 4.** TAZ M having more complex structure than TAZ protein.
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 the 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 to 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. Most boys with BTHS died during fetal life through to infancy from either heart failure or overwhelming infection. 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. This contrasted with patients identified prospectively and managed proactively, for whom mortality had fallen to just 10% 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.

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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. Recently, CL has been shown to be critical for the correct organization of ATP synthase assembly. Mutations have been reported in all exons of TAZ, including a variant of unknown significance in exon 5. 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 the 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 to 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.

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