A Novel Alu-Mediated Xq28 Microdeletion Ablates *TAZ* and Partially Deletes *DNL1L* in a Patient With Barth Syndrome

Harinder R. Singh,[1] Zhao Yang,[2] Saad Siddiqui,[1] Liana S. Peña,[2] Brandy H. Westerfield,[2] Yuxin Fan,[2] Jeffrey A. Towbin,[2] and Matteo Vatta[2]*

[1]Children's Hospital of Michigan, Division of Cardiology, The Carman and Ann Adams, Department of Pediatrics, Wayne State University School of Medicine, Detroit, Michigan

[2]Department of Pediatrics (Cardiology) and John Welsh Cardiovascular Diagnostic Laboratory, Baylor College of Medicine, Texas Children's Hospital, Houston, Texas

TO THE EDITOR:

Barth syndrome is characterized by X-linked cardiomyopathy, neutropenia, skeletal myopathy, growth delays, lipid abnormalities and increased excretion of 3-methylglutaconic acid [Barth et al., 1983; Kelley et al., 1991]. The locus causing Barth Syndrome was mapped to Xq28 and the tafazzin or *TAZ* gene was identified in 1996 [Bione et al., 1996]. Since then, more than 90 mutations in the *TAZ* gene have been identified according to the Barth Syndrome Foundation (www.barthsyndrome.org), which include frameshift, duplication, nonsense and missense mutations [Johnston et al., 1997; Brady et al., 2006]. There is no strong genotype-phenotype correlation, with patients presenting with a variable course of the disease [Gedeon et al., 1995; Barth et al., 2004]. Cardiac involvement, usually mild to moderate, occurs during the first year of life and presents as dilated cardiomyopathy (DCM), endocardiofibroelastosis (EFE), or isolated left ventricular non-compaction (LVNC) [Spencer et al., 2006]. DCM is more common and is characterized by left and right ventricular dilation, with the left chamber being the more severely involved, while septal and left ventricular (LV) wall thickness can also occur. Cardiac hemodynamics in Barth syndrome subjects usually demonstrates moderately reduced ejection fraction (EF), fractional shortening (FS) and increased left ventricular internal dimension in diastole (LVIDd) [Spencer et al., 2006].

In this study, we analyzed a 7-month-old previously healthy African American male, admitted to the hospital in moderate respiratory distress, congestive heart failure (CHF), and unremarkable family history. Two-dimensional echocardiogram revealed a dilated left ventricle (LV), FS = 10%, and EF = 22% consistent with DCM without evidence of hyper-trabeculated LV, great arteries or coronary arteries anomalies. He had normal fatty acid, vanilmandelic acid (VMA) and amino acid levels, while 3-methylglutaconic acid levels were increased. In addition to the cardiovascular and metabolic defects, the 7-month-old patient also

presented with neurological alterations including recurrent seizures and hypotonia.

After a few months from discharge, the patient was readmitted to the hospital due to hypoglycemia and deteriorating CHF despite optimal medical therapy. Due to his worsening hemodynamic conditions he underwent mechanical support through a Berlin Left Ventricular Assistive Device implantation followed by Extra Corporeal Membrane Oxygenation (ECMO). He was listed for heart transplantation. Prior to listing the patient for transplant, an extensive work up was performed. The electromyography and the nerve conduction study suggested skeletal myopathy and the muscle biopsy demonstrated non-specific changes with few scattered moderately atrophic fibers. The complete blood count (CBC) done during this admission (and on subsequent review of previous CBC reports) identified severe neutropenia showing a cyclical pattern ranging from a neutrophil count of 100 cells/mm³ to

Harinder R. Singh and Zhao Yang contributed equally to this work.
*Correspondence to:
Matteo Vatta, Pediatrics (Cardiology), Baylor College of Medicine, Texas Children's Hospital, 1102 Bates St., F.C. 430.04, Houston, TX 77030.
E-mail: mvatta@bcm.tmc.edu

14,700 cells/mm^3, which suggested the diagnosis of Barth syndrome. The patient underwent an orthotropic cardiac transplantation at 2 years of age and pathological analysis on the explanted heart, which weighed 128 g, identified DCM with disorganized myocytes, focal hypertrophic changes, perinuclear myocytolysis and EFE affecting both ventricles with focal epicardial calcifications with foreign body giant cell reaction.

The hemodynamics of this patient demonstrates a FS of 41% with excellent contractility. The neutropenia continues to be an issue with the absolute neutrophil count ranging between 0 and 3,000 (predominantly < 1,500), his skeletal myopathy has been stable and his tone has improved with physical therapy showing no residual weakness. The patient is growing according to the normal parameters for his age. He is still followed by Hematology/Oncology and continues to have intermittent neutropenia but has not required admissions for these episodes. He is also followed by a neurologist because of previously recurrent seizures that required treatment with Trileptal. However, the patient has not presented with seizure episodes for about a year.

Blood was obtained after informed consent, according to the Baylor College of Medicine Institutional Review Board (IRB), for DNA extraction. Using polymerase chain reaction (PCR) and direct DNA sequencing, open reading frame/splice site mutational analysis was performed on the *TAZ* gene (NCBI# NM_000116). PCR products were purified using exonuclease I (USB, Cleveland, OH)

and shrimp alkaline phosphatase (Roche, Indianapolis, IN). DNA sequence analysis was performed using Big Dye terminator chemistry (v3.1) and an ABI3730 genetic analyzer (Applied Biosystems, Foster City, CA). The comprehensive mutational analysis failed to yield a detectable product for all 11 exons of the *TAZ* gene. Therefore, a genomic DNA deletion was suspected. In order to map the potential breakpoints on the *TAZ* gene, sequential PCR was performed using primers upstream and downstream the coding sequence. Long fragment PCR product was obtained using primers flanking the deleted region: 5′-TGGCTGCACACTTCTGAGTT-3′ and 5′-AGGTGGGGAATTGCTAGGTC-3′. Amplified PCR product was purified and directly sequenced as described above.

Sequential PCR primers followed by junctional fragment PCR located the breakpoints 1.1 kb upstream (5′-UTR) and 12.6 kb downstream (3′-UTR) the start codon (ATG) of the *TAZ* gene. Computational analysis of the *TAZ* genomic sequence encompassing the breakpoint identified Alu-repeat elements of distinct subfamilies in both 5′- and 3′-UTR regions of *TAZ*. The breakpoint sequence identified a 29-bp core motif (Fig. 1A) with 100% homology to an AluSx-repeat at the 5′-UTR and one AluSq-repeat element at the 3′-UTR of *TAZ* (Fig. 1B). Alu-mediated microdeletion due to homologous unequal recombination is a very well described mechanism [Batzer and Deininger, 2002], supported by the high homology between the two Alu-elements involved (>78%). In addition, successful amplification of adjacent microsatellite markers (Table I) and the *Emerin* (*EMD*) gene, which is
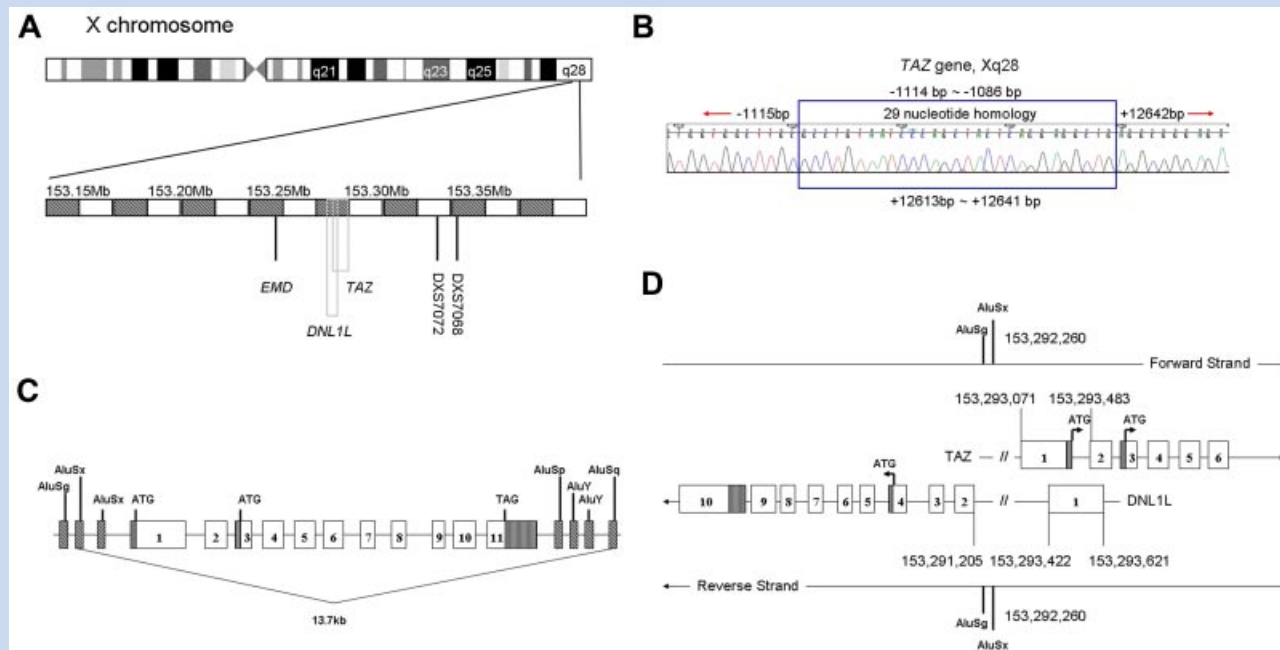
FIG. 1. A: Chromosomal deletion study using microsatellite markers and *Emerin* (*EMD*) gene in Xq27–28 to map the deletion. Successfully amplified products are defined by the black solid bar, while markers that failed to amplify are labeled as red squares. B: The sequence at the joining of the deletion ends and *TAZ* gene location in Xq28. There is a 29-nucleotide homology between the 2 breakpoints. C: Relative location of the full-length Alu-repeat elements relative to the 5′- and 3′-UTR of the *TAZ* gene. D: Relative chromosomal position, expressed in base pairs, of the 5′ AluSx-repeat involved in the microdeletion and the overlapping *TAZ* and *DNL1L* exonic and intronic sequences. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I. Panel of Polymorphic Markers and Gene Analysis on Chromosome X

Marker name	Physical location (bp)	Cytogenetic location	Amplified
DXS8040	66963873	Xq12	Yes
DXS1050	87160632	Xq21.31	Yes
DXS8097	105909211	Xq23.3	Yes
DXS8055	114561198	Xq24	Yes
DXS8093	125610089	Xq25	Yes
DXS8041	133531399	Xq26.3	Yes
DXS984	139459394	Xq27.1	Yes
DXS1227	140630081	Xq27.2	Yes
DXS998	150167607	Xq27.3	Yes
DXS8061	151772275	Xq28-tel	Yes
EMD	153260751	Xq28-tel	Yes
DNL1L	153293621	Xq28-tel	Partially
TAZ	153293071	Xq28-tel	No
DXS7072	153333143	Xq28-tel	Yes
DXS7068	153341684	Xq28-tel	Yes
DXS7010E	153382680	Xq28-tel	Yes

located 20 kb upstream the *TAZ* gene (Fig. 1B) suggested that the recombination event did not impact other chromosomal regions.

However, careful analysis of neighboring genes revealed that the Alu-mediated chromosomal deletion involved the 5′-UTR, the first exon and partial intron1 of three out of six isoforms of the Deoxyribonuclease I-like 1 Precursor (*DNL1L*) gene (www.ensembl.org; ENSG00000013563), which is transcribed on the opposite strand (3′>5′) and it partially overlaps with *TAZ*. *DNL1L* is highly expressed in cardiac and skeletal muscle, although very little is known about its function and it has never been involved in Barth syndrome before. DNL1L was shown to localize to the plasma membrane and to early endocytic vesicles to hydrolyze the internalized extracellular DNA, thereby protecting cells from invasion by foreign genes [Shiokawa et al., 2007]. In addition, a deletion polymorphism has been studied for probable association with acid maltase deficiency disorders and Pompe disease [Malferrari et al., 2001], although this association appears controversial [Lichtenbelt et al., 2006]. Unfortunately, we could not obtain consent to acquire myocardial tissue from our patient to investigate the involvement of DNL1L in the clinical presentation of our subject and whether the Alu-mediated deletion led to alterations in DNL1L expression. Therefore, because of the extent of the deletion, we can only speculate that, at least three out of six DNL1L isoforms would not be expressed in our patient's heart consistent with a probable partial functional ablation in addition to the null *TAZ* mutation. Further studies on DNL1L function are warranted to determine its significance in cardiac biology and its possible involvement in Barth syndrome.

Clinically, our patient demonstrated a very severe cardiomyopathy with rapid progression requiring cardiac transplantation, cyclical neutropenia with very low counts, dramatic hypotonia and weakness. Genetically, we have identified the first chromosomal microdeletion (13.7 kb on Xq28) due to an Alu-mediated unequal homologous recombination involving the entire *TAZ* gene and partially the *DNL1L* gene overlapping on the opposite strand. The microdeletion included part of the 5′-UTR, the entire coding sequence and a fragment of the 3′-UTR of *TAZ*, all the sequence upstream the first exon and part of the intron 1 of *DNL1L*, confirming that the Xq28 is an unstable region of the human genome prone to chromosomal rearrangements as demonstrated by diseases such as incontinentia pigmenti, caused by 878 bp MER67B repeats-mediated deletion of the *NEMO* gene [Aradhya et al., 2001], or hemophilia-A caused by a ∼500 kb inversion disrupting the *FVIII* gene via an *int22h* repeat-mediated homologous recombination [Lakich et al., 1993; Naylor et al., 1993]. In addition, the fragile site FRAXE (*FMR2*) is located on chromosome Xq28, approximately 600 kb distal to another fragile site FRAXA (*FMR1*) on Xq27.3, which is responsible for fragile X syndrome [Chastain et al., 2006].

Therefore, the Alu-mediated deletion involving both TAZ and DNL1L present in our patient provides further evidence for the instability of the Xq28 region of the X chromosome and broadens the range of genomic alterations observed in Barth syndrome, thus increasing the complexity of the genetic testing for Barth patients.

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