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Letters to Editor

Targeted next generation sequencing reveals novel splice site mutations in *COL6A3* gene in a patient with congenital muscular dystrophy

Sir,

A 4.5-year old female child of nonconsanguineous marriage was diagnosed as a case of congenital muscular dystrophy (CMD) on the basis of clinical findings, neuroimaging, mildly elevated creatine phosphokinase levels (279 IU/L), and immunohistochemistry studies on muscle tissue [Figure 1]. There was generalized hypotonia, diminished deep tendon reflexes, and predominantly proximal muscle weakness. There was marked laxity at the wrists and metacarpophalangeal joints and fixed flexion contractures at both the knees. Electroneuromyography was suggestive of primary muscle disease, and muscle biopsy showed nonspecific features [Figure 1a-f].

The targeted gene panel sequencing, interrogating 80 known congenital myopathy and muscular dystrophy candidate genes, was performed on Illumina sequencing platform for molecular diagnosis, which identified a novel *de novo*



Figure 1: Histopathological findings:(a and b) Maintained fascicular architecture with few atrophic fibers. (c and d) ATPase at pH 9.4 shows type I fiber predominance and atrophy. (e) Nicotinamide adenine dinucleotide (NADH) is negative for central cores. (f) Modified Gomori's trichrome staining (mGT) is negative for nemaline rods



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Figure 2: Representative Sanger sequencing chromatogram of c.6283-2A>C mutation (a. Control (A/A), b. Patient (A/C), c. Father (A/A) and d. Mother (A/A). The double peaks observed in the patient (b) just after the SCV000245346.1 mutation (marked with red arrow) also confirms the presence of heterozygous 12 bp insertion (c.6283-1->CTGGGCTCTCCT) at the same 3' splice junction



Figure 3: Schematic diagram and Sanger sequencing chromatogram of c.6283-2A>C mutation analysis in cDNA from the patient, along with the result of reverse transcriptase polymerase chain reaction (RT-PCR) from the patient's cDNA, analyzed on 2% agarose gel electrophoresis. Lane 1 contains the 232 bp band of the control, lane 2 represents the heterozygous 232/205 bp bands of the patient, which indicates the presence 27 bp deletion (exon 18) in the patient. Sequencing revealed that amplicon from the patient's cDNA (reverse strand) was missing the entire exon 18 sequence due to the c.6283-2A>C splice site mutation. The double peaks present in the chromatogram indicate the heterozygous status of the deletion

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heterozygous splice variation (NM_004369.3:c.6283-2A>C) in intron 17 of the COL6A3 gene along with an intronic insertion of 12 bp sequence (c.6283-1->CTGGGCTCTCCT) at the same 3' splice junction [Figure S1]. These variations are not reported in the 1000 genomes, Exome Variant Server, Exome Aggregation Consortium, and the Single Nucleotide Polymorphism dbSNP databases, and the region is conserved across primates. The NGS data analysis pipeline was described earlier.^[1] Sanger sequencing of the amplicons using ABI 3130 Genetic analyzer (Life Technologies, CA, USA) confirmed these mutations in the patient and a homozygous normal status in both the parents [Figure 2]. The c.6283-2A>C mutation was assessed using web-based programs for analysis of potential splicing aberrations,^[2,3] all of which scored low for the mutant splice site [Table S1 and Figure S2].

Further functional analysis using complementary deoxyribose nucleic acid (cDNA), synthesized by priming of 1 µg total ribose nucleic acid (RNA) using SuperScriptTM III First-Strand cDNA Synthesis System (Invitrogen, Carlsbad, CA) followed by polymerase chain reaction (PCR) around the mutation and bidirectional Sanger sequencing revealed absence of complete exon 18 of COL6A3gene in the patient [Figure 3]. The exon 18 skipping retains the reading frame and is predicted to introduce a 9 amino acid deletion in the triple helix (TH) domain of the protein, which is predicted to serve as a cell attachment site as a part of type VI collagen.^[4] In all Col6 proteins, the TH domain is the major contributor to the shape of the secondary structure. In addition, the region is highly conserved across the species. It is possible that these splice-site mutations, deleting the whole exon 18 (equivalent to 9 amino acids) from this important TH domain of the protein, has a damaging influence on the structure and function of the alpha 3 type VI chain of collagen, which ultimately may lead to the affected phenotype of the patient.

Finally, to conclude, in the present study, we report two simultaneously occurring potentially pathogenic novel mutations–a *denovo3'* splice-site c.6283-2A>C point mutation and a c.6283-1->CTGGGCTCTCCT insertion in the *COL6A3* gene, detected by targeted gene panel sequencing, in a patient diagnosed with CMD. Targeted NGS test results helped us to diagnose the patient as Ullrich Congenital Muscular Dystrophy type 1 (OMIM #254090). We have reported the c.6283-2A>C variant to ClinVar (SCV000245346.1).

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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Aneek Das Bhowmik, Karthik Tallapaka¹, Megha Uppin², Challa Sundaram², Ashwin Dalal

Diagnostics Division, Centre for DNA Fingerprinting and Diagnostics, Departments of ¹Medical Genetics and ²Pathology, Nizam's Institute of Medical Sciences, Hyderabad, Telangana, India

Address for correspondence:

Dr. Ashwin Dalal, Diagnostics Division, Centre for DNA Fingerprinting and Diagnostics, Inner Ring Road, Uppal, Hyderabad - 500 039, Telangana, India. E-mail: adalal@cdfd.org.in

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