A Novel Nonsense Mutation in FERMT3 Causes LAD-III in a Pakistani Family

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INTRODUCTION

Leukocyte adhesion deficiency (LAD) is a primary immunodeficiency disorder caused by a defect in neutrophil adhesion to the vessel endothelium. There are three different types of this disease, and LAD3, also known as LAD1 variant (LAD1V), is the most rare form (AlmarzaNovoa et al., 2008). Additional manifestations of this disease include bleeding diathesis similar to what occurs in the Glanzmann thrombasthenia, which can, however, be excluded by normal platelet aggregation tests (Boudreaux et al., 2010). LAD3 is caused by a genetic defect in the FERMT3 gene. This defect...
leads to abnormal expression of kindlin-3, a protein whose major role is the regulation of integrin activation, which is essential for the adhesion of leukocytes and platelets (Robert et al., 2011).

Genetic mutations in the FERMT3 gene (OMIM 607901) run in autosomal recessive pattern in LAD-3 (OMIM 612840) families. FERMT3 also known, as KIND3, MIG2B, UNC112C, URP2, or URO2SF, is located on chromosome 11q13.1. It encodes kindlin-3, a cytoskeleton protein involved in the stabilization and activation of the glycoprotein receptor integrin through attachment to its beta subunit. These interactions are responsible for maintaining a stable integrin conformation and to activate its subunits (Ley et al., 2007). Genetic alterations in the FERMT3 gene cause disruption of the adherent property of integrin on both leukocytes and platelets, possibly due to defect in integrin; its structure is intact, but activation (and thus binding) is not appropriate (Svensson et al., 2009; Zimmerman, 2009).

LAD3 and LAD1 have similar clinical manifestations i.e., leukocytosis, delay in the detachment of the umbilical cord, and critical life-threatening bacterial infections. In addition, there is platelet aggregation dysfunction, which results in severe bleeding episodes. This disorder has mostly been reported in patients of Turkish, Arab Maltese or African American origin. In the present study, we used targeted next-generation sequencing (TGS) technology, the advance methodology (Zhu et al., 2017), and found a novel homozygous mutation in the FERMT3 gene in a Pakistani family with autosomal recessive LAD3. Sanger sequencing-based prenatal diagnosis was offered to the family for the successive pregnancy, and it confirmed the co-segregation of this genetic mutation with the phenotype in this family.

**CASE PRESENTATION**

**Clinical Report**
The index patient is a seven-month-old boy born to first cousins parents, presenting with a prolonged history of fever and recurrent infections for 4 months. Parents reported intermittent bleeding episodes from the nose, mouth, and anus that, during patient hospitalization, were unsuccessfully treated with broad-spectrum antibiotics and packed red cells and platelets transfusion. Examination revealed a failure to thrive in the child, with both height and body weight below the 3rd percentile. He had severe pallor, bruises all over the body, and there were bilateral anterior and posterior cervical palpable lymph nodes, which were firm and tender. The liver was also palpable; it was 9 cm in span, soft and non-tender, while a firm spleen was also palpable 3 cm in its longitudinal axis. The previous record had shown bcytopenia and leukocytosis, growth of multiple microorganisms in blood, including *Burkholderia cepacia* and *Staphylococcus aureus*, and persistently high inflammatory markers. Extensive investigations done during this admission confirmed the anemia, thrombocytopenia, and leukocytosis. Bone marrow aspiration and trephine biopsy showed cellular marrow. Basic primary immunodeficiency workup showed normal immunoglobulin, while flow cytometry revealed normal CD18 expression. There was strong suspicion of primary immunodeficiency due to the persistent leukocytosis and recurrent infections.

**METHODS**

**Ethics Statement, Consent Statement, and Proband**
The study protocol was in accordance with the Institutional Review Board (ERC/IRB) and conformed to the tenets of the Declaration of Helsinki. Written informed consent was obtained from the parent of the patient for the publication of this case report. This study consisted of the proband and three closely related family members from two generations, with history of consanguineous marriage, described by their genetic workup and pedigree analysis in Figure 1.

**Targeted Next Generation Sequencing**
Peripheral blood samples of the family were drawn and DNA was extracted using a QIAamp DNA Blood Mini Kit (Qiagen), following manufacturer’s instructions. The targeted next generation sequencing was performed using the Illumina TruSight™ Inherited Disease sequencing panel, a disease targeted sequencing research panel focusing on 552 genes in regions known to harbor recessive pediatric pathogenic mutations. This panel targets 2.25 Mb of the human genomic content, with fragments of ∼500 bp. The medium coverage of the sample was >95% of amplicons at >100× coverage. Library was constructed by capturing targeted region using TruSight™ rapid capture. Enriched libraries were loaded onto flow cell (Illumina, CA, United States) and paired-end sequencing runs were processed on a MiSeq (Illumina™) genome sequencer. Data analysis alignment was performed with on-instrument MiSeq reporter software. The mutations identified as pathogenic were confirmed using Sanger method according to the standard protocol (BigDye® Terminator v3.1 Cycle Sequencing Kit, Applied Biosystems™).

**Sanger Sequencing**
Polymerase chain reaction (PCR) amplification and Sanger sequencing of the TGS-identified variant was performed to confirm TGS results. Primers listed in Table 1 surrounding the identified mutation were used to amplify a product of 332 bp, which was then sequenced by Sanger sequencing on an ABI-3500 sequencer instrument (Applied Biosystems Inc., Foster City, CA, United States).

**RESULTS**

**Mutation Screening by Targeted Next Generation Sequencing**
The identification of the severe immunodeficiency-causing gene mutations, through targeted inherited diseases sequencing panel, was performed on the index patient gDNA sample. Disease causing mutations were identified by the VariantStudio software
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FIGURE 1 | Pedigree with LAD-3 diseases in the index patient. The patient (II-1) indicates the homozygous nonsense variant of the FERMT3 gene in an electropherogram: NM_178443:c.286C>T and CVS (II-2) indicate with nonsense mutation as heterozygous carrier.

and Variant Interpreter tool (Illumina). These interpretation modules can call variants automatically; options are available to apply stringent filters and for the annotation of NGS data (Hu et al., 2017). The in silico prediction tools SIFT, Polyphen 2, MutationTaster, MutationAssessor, dbSNP, and COSMIC were applied to filter pathogenic, benign and variant of uncertain significance. Some of the newly identified variants were not present in any of the above-mentioned public databases, and would need further verification. Interestingly, a single homozygous nucleotide substitution (c.C286T) in the exon 3 of the FERMT3 gene (NM_178443) was identified in this index patient. This mutation leads to an amino acidic change from glutamine (Gln, Q) to a stop codon in position 96 of the kindlin-3 protein (p.Q96*). Additional details of the identified sequence variant c.C286T (p.Q96*), together with associated pathogenic effects, are mentioned in Table 2 and Figure 1. This variant was seemed to be novel, as it could not be identified by searching in other databases like dbSNP, COSMIC, and HGMD (Table 2). Variants identified in other genes for EVC, DPYD, COL4A3, and TSPYL1 by NGS were excluded as not having a damaging effect assessed by prediction tools on proteins. Finally, this family was offered prenatal diagnosis during subsequent pregnancy, which was performed by chorionic villous sampling done during the 11th week of gestation and Sanger sequencing; the fetus was found to be heterozygote for the same mutation c.C286T (p.Q96*) (Figure 1).

DISCUSSION

Leukocyte adhesion deficiency-III (LAD3) is a rare and recently identified primary immunodeficiency, which has different genetic mutations than the ones present in the other two LAD types. In the current study, we found a novel homozygous, stop codon variant c.C286T (p.Q96*) in the FERMT3 gene in a Pakistani family. The protein structure of FERMT3 comprises of a FO

| Primer | Sequence 5’-3’ | Product size | TM |
|--------|----------------|--------------|----|
| Forward (FERMT3-F) | CTGAATCCTGGGTTGTGCT | 332 bp | 62°C |
| Reverse (FERMT3-R) | GAATCAGCGGGCAGACTTAC | | |

TABLE 2 | FERMT3 variant identified in a LAD3 patient.

| Gene | Exon | Nucleotide | Protein | Type | Status | dbSNP |
|------|------|------------|---------|------|--------|-------|
| FERMT3 | 00 | c.C286T | p.Q96* | Homozygous | Nonsense | Novel |

FERMT3, leukocyte adhesion deficiency-III; c, variation at cDNA level; p, variation at protein level; * stop codon.
Robert et al. (2011) reported a p.N54Rfs142 mutation identified at the N-terminal of the protein, specifically in the FO domain that have the binding site to integrin beta subunit. Similarly, nonsense mutations also lying within the FO domain. In vitro studies revealed that this mutation was causing a decrease in the mRNA level resulting in an unstable transcript. The nonsense mutation p.Q96X that we identified in this study is also lying within the FO domain. Similarly, nonsense mutations leading to defects in protein expression were reported in patients of Turkish (Mory et al., 2008; Kuijpers et al., 2009; Svensson et al., 2009), Arab (Kuijpers et al., 2009; Malinin et al., 2009), Maltese (Svensson et al., 2009), and African American origin (McDowall et al., 2010).

To date, very few cases have been described for Leukocyte Adhesion Deficiency III; most of the affected individuals (323) were diagnosed with LAD1 (AlmarzaNovoa et al., 2008), while LAD-3 seems to be more sporadic. It is possible that LAD is reported with even lower frequency, due to the failure in correctly diagnosing rare entities. LAD3 cases caused by genetic mutations in FERMT3 were reported in Turkish and Maltese patients; a homozygous nonsense mutation (R509X) was reported in the Turkish patients, whereas expression was normal in their parents. A novel p. R573X nonsense mutation in FERMT3 was reported in a Turkish patient, while p.W229X in Arabic patients. In vitro studies revealed that FERMT3 protein was not present in leukocytes and platelets of all tested patients, which had, however, similar defects in neutrophil and platelet function (Kuijpers et al., 2009). In addition to its adhesion properties, FERMT3 gene product is also involved in leukocyte migration. This was confirmed by the in vitro effects of the homozygous mutations (G308R and 1275delT) in the FERMT3 gene, which were the cause of severe LAD3 in an African American girl (McDowall et al., 2010). Almost all cases of LAD III were diagnosed with innate immune defects. However, Suratannon et al. (2016) identified p.Gln599Ser mutation in FERMT3 gene in Thai patient that presented with humoral immune defect (Suratannon et al., 2016). Table 3 summarizes all the mutations in the FERMT3 identified in the literature. To the best of our knowledge, this FERMT3 variant is a novel mutation that broadens the mutation spectrums of LAD3. Thus, this finding shows that the recessive FERMT3 mutation c.C286T (p.Q96*) likely caused LAD-3 in our studied Pakistani pedigree.

**CONCLUSION**

In conclusion, this study wants to stress the importance of early diagnosis. As in the majority of primary immunodeficiency diseases, the prognosis of LAD3 is extremely dependent on early age diagnosis, with timely management of bacterial infections and consideration for HSCT. In addition, this autosomal

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**TABLE 3 | List of reported FERMT3 mutations in LAD3.**

| S. No | Nucleotide change | Protein change | Type | References |
|-------|------------------|----------------|------|------------|
| 1     | c.286C > T       | p.Gln96X       | Nonsense | Current study |
| 2     | c.1069C > T      | p. Arg357X     | Nonsense | Wolach et al., 2019 |
| 3     | c.1795C > T      | p. Gln599Ser   | Missense | Suratannon et al., 2016 |
| 4     | c.1597C > T      | p. Gln33X      | Nonsense | Crazzolara et al., 2015 |
| 5     | c.1426C > T      | p. Gln476X     | Nonsense | Harris et al., 2012 |
| 6     | c.238_244dup     | p.Lys82ThrfsX67 | Insertion | Van de Vijver et al., 2012 |
| 7     | c.92G > A        | p. Gly308Arg   | Missense | McDowall et al., 2010 |
| 8     | c.1275delT       | p.Glu426ArgfsX3 | Frame shift | McDowall et al., 2010 |
| 9     | c.161-2A > C     | p.Asns4ArgfsX142 | Splice site | Robert et al., 2011 |
| 10    | c.687G > A       | p.Trp228X      | Nonsense | Manevich-Mendelson et al., 2009; Robert et al., 2011 |
| 11    | c.48G > A        | p.Trp16X       | Nonsense | Malinin et al., 2009 |
| 12    | c.1671-2A > G    | p.Deletion exon 14, p.Phe555TrpsX141 | Splice site | Svensson et al., 2009 |
| 13    | c.1729C > T      | p.Arg577X      | Nonsense | Kuijpers et al., 2009 |
| 14    | c.1717C > T      | p.Arg573X      | Nonsense | Kuijpers et al., 2009; Jurk et al., 2010 |
| 15    | c.1525C > T      | p.Arg509X      | Nonsense | Mory et al., 2008; Kuijpers et al., 2009; Malinin et al., 2009; Svensson et al., 2009 |
| 16    | c.1537C > T      | p.Arg513X      | Nonsense | Mory et al., 2008; Kuijpers et al., 2009; Malinin et al., 2009; Svensson et al., 2009 |

FERMT3, leukocyte adhesion deficiency-III; c, variation at cDNA level; p, variation at protein level.
recessive disorder has high incidence in areas with high rate of consanguineous marriages. Therefore, broadening the spectrum of known mutations underlying the phenotype of such a life-threatening disease can help offering and performing better genetic counseling and prenatal diagnosis.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations Institutional Review Board (ERC/IRB). The protocol was approved by the Institutional Review Board (ERC/IRB). Written informed consent was obtained from the parents of the subjects in accordance with the Declaration of Helsinki.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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