LETTER TO THE EDITOR

Adaptive immune defects in a patient with leukocyte adhesion deficiency type III with a novel mutation in *FERMT3*

To the Editor,

Leukocyte adhesion deficiency (LAD) is a rare primary immunodeficiency disease characterized by impairment of phagocyte adhesion (1–3). Three subtypes have been classified by distinct phases of the adhesion cascade. LAD-III is caused by defects in signaling pathways used for integrin activation in all hematopoietic cell types leading to recurrent infections with poor platelet aggregation resembling Glanzmann's thrombasthenia (4). Mutations in *FERMT3* have been identified to underlie LAD-III (4, 5). *FERMT3* encodes kindlin-3, one of the focal adhesion proteins which contain a FERM domain located at the carboxyl terminus binding to β-integrin cytoplasmic tails. This molecule cooperates with the cytoskeletal protein talin leading to integrin activation. It also stabilizes active conformations of the integrin subunits and the ligand binding (5, 6). Evidently, integrins are widely expressed in many cell types including T and B lymphocytes. Defects in integrin function therefore could lead to both innate and adaptive immune dysfunctions. However, almost all reported cases of LAD-III only had innate immune defects. Here, we describe a female Thai patient who was diagnosed with LAD-III, yet presenting with a mild atypical phenotype in which a humoral immune defect was detected.

Our patient was the second child of consanguineous parents who were first cousins. The pedigree of the family is shown in Fig. 1a. She presented with early-onset severe gram-negative infections, thrombasthenia, hepatosplenomegaly, and defective wound healing. Between three and 8 months old, she experienced four episodes of bacterial pneumonia with sepsis. Firstly, she had severe pneumonia and subsequently developed acute respiratory distress syndrome. Cultures of tracheal suction specimens revealed *Acinetobacter baumannii*. *Salmonella* spp. was also reported from stool samples when she was found to have diarrhea. In the second episode of pneumonia, *A. baumannii* was reported again from specimens obtained by tracheal suctioning. *Pseudomonas aeruginosa* was isolated from ear discharge. Thirdly, the patient had pneumonia with septic shock. Tracheal suction cultures revealed *Streptococcus mitis* and *Escherichia coli*. Finally, necrotizing pneumonia was reported. The patient's blood culture was positive for *P. aeruginosa*. She had the ability to form pus, although minimal, and umbilical cord separation occurred at the age of 9 days. After prolonged courses of antibiotics, the patient had developed mucocutaneous candidiasis. She did not suffer from invasive fungal infections, as described in other patients with LAD-III (3, 7). Her bleeding symptoms were mild and appeared only during episodes of infections, while spontaneous intracranial bleeding and/or massive pulmonary hemorrhage have been reported in patients with typical LAD-III (3, 7). Initial investigations and immunologic assessment at the age of 5 months revealed persistent leukocytosis with neutrophilia, anemia, and thrombocytopenia. A complete blood count showed a hematocrit of 28% (29–42), white blood cell count of 45,430 cells/mm^3 (6000–17,500), neutrophils of 25,440 cells/mm^3 (4000–12,000), lymphocytes of 10,903 cells/mm^3 (2000–17,000), and platelets of 109,000 cells/mm^3 (300,000–700,000). Flow cytometric analysis of lymphocyte populations demonstrated normal numbers of total T cells (CD3+), CD4+ T cells, CD8+ T cells, B cells (CD19+), and NK cells (CD16+56+).

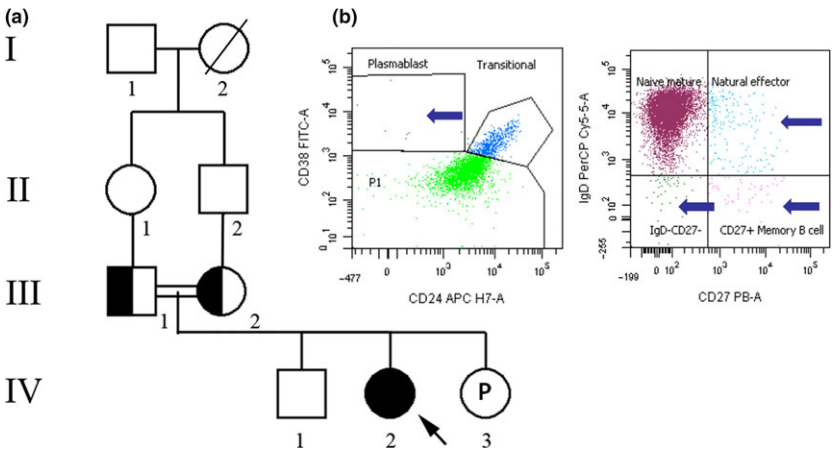

Figure 1 (a) Pedigree. (b) Flow cytometry revealed low proportions of class-switching memory B cells (CD27+IgD−), marginal zone-like cells (CD27+IgD+IgM+), CD27− memory B cells (IgD-CD27−) and plasmablasts (CD24-CD38hi) (arrows), for the exact proportions see text.

Analysis of lymphocyte subpopulations showed low percentages of class-switched memory B cells (CD27+IgD-IgM−) 0.5% (0.9–29), marginal zone-like B cells (CD27+IgD+IgM+) 1.7% (2–43), CD27− memory B cells (CD27−IgD−) 0.5% (1.6–3.6), and plasmablasts (CD24-CD38hi) 0.1% (0.1–4) (Fig. 1b). The proportion of CD4+ terminally differentiated effector memory T cells (Temra) was also reduced to 4.5% (<9.5). The proportion of CD11b-positive neutrophils was comparable to healthy controls; 30.2 vs. 37.8%. Immunoglobulin (Ig) levels were measured at the age of 5 months; IgG was in the lower normal range (2.85 g/l, normal range 2.41–6.13). Serum IgM/IgA/IgE (0.64, 0.1, <0.04 g/l) were within normal ranges. Lymphocyte proliferation stimulated by phytohemagglutinin, PPD antigen, and tetanus antigen were normal compared with healthy controls. Anti-HBs after vaccination was low (28.7 mIU/ml) while the protective value was more than 10 mIU/ml). The level of rabies virus neutralizing antibody after a three-dose regimen (day 0, 3, 7) of purified vero-cell rabies vaccine was markedly reduced compared to controls measured by a rapid fluorescent focus inhibition test (1.75 vs. 7.57 IU/ml). X-rays of the extremities showed an increased bone density (osteopetrosis). Platelet aggregation studies using G protein-coupled receptor (GPCR) agonists (ADP, collagen, and arachidonic acid) showed completely absent aggregation. Notably, ristocetin stimulation revealed normal platelet aggregation.

Regular intravenous immunoglobulin (IVIG) therapy because of a suspected humoral immune defect was started at the age of 11 months. Thereafter, she had only one urinary tract infection at the age of 15 months. IVIG was discontinued when she was 19 months old as she was in stable condition. Six weeks after IVIG discontinuation, she developed gross hematuria, urinary tract infection, and severe sepsis requiring intensive care unit admission. Her IgG, IgM, and IgA levels were low; 4.22 (5.53–9.71), 0.19 (0.35–0.81), and 0.10 g/l (0.26–0.74), respectively. IVIG was reintroduced from the age of 21 months onwards. No severe infections occurred up to her last follow-up at the age of 3 years.

After informed consent, the patient's and the parents' genomic DNA were extracted from peripheral blood leukocytes using AchivePure DNA Blood Kit 5 PRIME Inc., Gaithersburg, MD, USA. Whole-exome sequencing was performed and revealed a homozygous novel missense mutation in *FERMT3* (11:63990633; hg19) at nucleotide position 1784, changing A to C (c.1784A>C). This mutation resulted in a change of codon 595 (p.Q595P) (Fig. 2a). Both parents were heterozygous for this mutation. Several lines of evidence indicate that the mutation is pathogenic. It changes the amino acid from a hydrophilic neutral glutamine to a hydrophobic proline. Furthermore, it is in the F3 subdomain, which appears to be functionally important in molecular interactions of FERMT3 with integrin β-subunit cytoplasmic tails (5). The mutation is located at an evolutionarily conserved residue found in other species using ClustalX program (Fig. 2b) and is predicted to be probably damaging by PolyPhen-2 (score of 0.995). The mutation was absent in 50 unaffected Thai controls. In addition, immunoblot showed absent FERMT3 protein (Fig. 2c).

Our case represents the first patient with a homozygous missense mutation in *FERMT3*. So far, only one other LAD-III patient carrying a missense mutation in *FERMT3* has been reported (8). However, this case was compound heterozygous for a missense mutation (c.922G>A, p.Gly308Arg) and a frameshift deletion (c.1275delT, p.Glu426ArgfsX3). Most previously reported mutations were non-sense or frameshift mutations (8). This could explain the milder manifestations in our patient compared to previously reported cases. We expected that our patient might have a small amount of FERMT3 protein with some remaining functions. However, the immunoblot using proteins extracted from her leukocytes

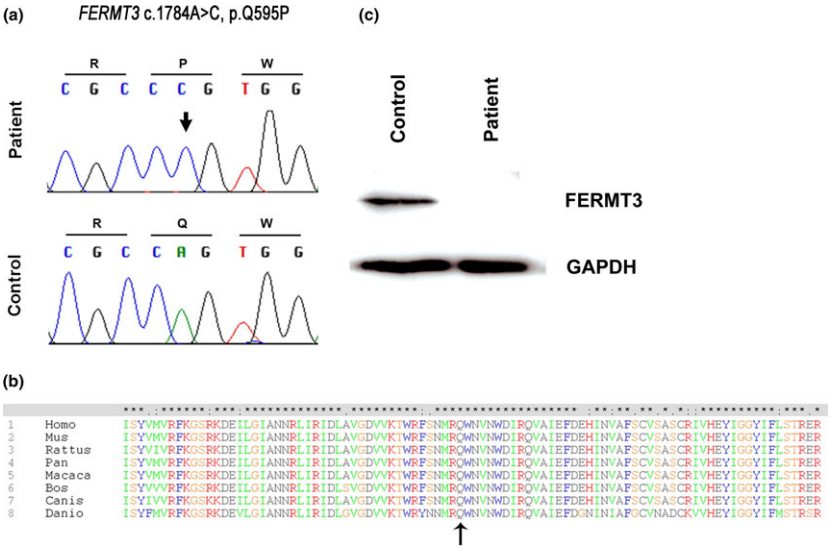

Figure 2 Mutation analysis of *FERMT3*. (a) Chromatogram of the patient (upper) and unaffected control (lower). (b) Multiple sequence alignment of FERMT3 in different species. (c) Immunoblot showing that FERMT3 protein was absent in our patient.

showed absence of FERMT3 protein expression. The reason for the milder phenotype remains to be elucidated.

There has been only one report from Robert et al. describing adaptive immune defects in LAD-III (9). A decreased lymphocyte proliferation in response to anti-CD3 antibody and a low immunoglobulin production was demonstrated (9). The patients described in this report were homozygous for a splice site mutation, c.310-2A>C (located before exon 3) while our patient had the mutation in exon 14. Adaptive immune dysfunctions have not been described in other LAD-III patients with mutations in exon 14 (8).

We hypothesize that the adaptive immune defects in LAD-III patients could be caused by the impairment of immunologic synapse formation between $CD4^+$ T cells and antigen-presenting cells as well as $CD4^+$ T cells and B cells, not from intrinsic B-cell defects. To prove this hypothesis, we performed *in vitro* stimulation of sorted naïve B cells from the patient and a healthy control using anti-IgM, anti-CD40, and IL-21 to evaluate immunoglobulin synthesis by ELISA and expression of surface beta-1 integrin in an active conformation (CD29) by flow cytometry. The results showed that the patient could produce IgG at 6.9 mg/ml/million cells (control 12.1 mg/ml/million cells) and IgA at 30.2 ng/ml/million cells (control 31.2 ng/ml/million cells). The surface expression of CD29 of the patient and control before and after stimulation was 9% vs. 10% and 27% vs. 61%, respectively, indicating a reduced CD29 expression on B cells.

When the B-cell receptor engages, intracellular protein kindlin-3 is activated and stabilizes the active conformation of the integrin which is required to sustain contact between T and B cells (9). Mice lacking kindlin/integrin binding had suboptimal B-cell numbers in lymph nodes and low antibody responses *in vivo* (10). B cells from our patient had an ability to produce antibodies *in vitro* but impaired antibody production *in vivo*. This may be explained by the fact that *FERMT3* mutations could cause a defect in integrin activation, leading to impairment of B-cell homing to lymph nodes and B-cell–T-cell interaction.

Impairment of B-cell and T-cell interaction might account for the low proportion of class-switched memory B cells. Marginal zone-like B cells and CD27- memory B cells, predominantly developing in a T-cell independent way, were also low, suggesting that at least a part of these cells could originate from the germinal center. This has been shown in patients with the class switch recombination defect CD40L deficiency, who also had low numbers of class-switching memory B cells and marginal zone-like B cells (11). However, why these features only occur in some patients with integrin defects remains elusive. We demonstrated that immunoglobulin therapy was able to reduce the severity and frequency of infections indicating that humoral immune defects could play a major role in the susceptibility to infection in this case. Whether IVIG therapy would benefit other LAD-III cases with normal immunoglobulin levels requires further studies.

In summary, we identified a LAD-III patient with a mild clinical phenotype, harboring a novel homozygous missense mutation in *FERMT3*. The patient had immunologic defects involving both innate and adaptive immune responses. Our study emphasized the importance of investigating adaptive immune function, particularly serum immunoglobulin levels, in addition to phagocytic functions in patients with LAD-III. The findings in our patient suggest that IVIG could be beneficial in LAD-III patients with low immunoglobulin levels. We propose that IVIG might be the treatment of choice in patients with a mild phenotype in the presence of a humoral immune defect or an adjunctive treatment in more severe cases, awaiting bone marrow transplantation as definitive therapy.

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