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A novel 3′ splice-site mutation and a novel gross deletion in leukocyte adhesion deficiency (LAD)-1 ☆

T.H. Bernard Cher [a], Hwee Sing Chan [a], Georg F. Klein [b], Jörg Jabkowski [b], Gabriela Schadenböck-Kranzl [c], Otto Zach [d], Xavier Roca [e], S.K. Alex Law [a,*]

[a] School of Biological Sciences, Nanyang Technological University, Singapore
[b] Elisabethinen Hospital, Department of Dermatology, Linz, Austria
[c] Department of Pediatrics, Hospital of Steyr, Steyr, Austria
[d] Elisabethinen Hospital, Department of Internal Medicine I, Linz, Austria
[e] Cold Spring Harbor Laboratory, NY, USA

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ABSTRACT

A patient was diagnosed with leukocyte adhesion deficiency-1. She was born in 1996 and her parents are not known to be related. Her leukocytes expressed less than 2% of the CD18 antigens relative to normal individuals. Molecular analysis revealed that she is a compound heterozygote. She inherited a 27,703 bp deletion from her father (g.43201_PTTG1IP:10890del27703), spanning from intron 11 of the gene for the β2 integrin (ITGB2, CD18, NG_007270.2) to intron 2 of the gene for the Pituitary Tumor-Transforming Gene 1 Interacting Protein (PTTG1IP, NC_000021.8). The maternal allele has a g.23457C>A mutation at position −10 in intron 2 of the ITGB2 gene, resulting in the activation of a cryptic 3′ splice site in intron 2 to include 43 intronic nucleotides (r.[59-43_59-1ins;59-10C>A]).

1. Introduction

The integrins are a family of type I transmembrane glycoproteins, expressed as obligate non-covalent heterodimers of integrin α and β subunits [1]. Integrins have critical roles in a wide range of biological processes including cell to cell interactions, cell to extracellular matrix interactions, cell migration, cell cycle control, cell survival, gene expression and rearrangement of the actin cytoskeleton [1–5]. Eighteen different α subunits and eight different β subunits associate into 24 distinct combinations of integrin heterodimers in humans. Among these, the β2 integrins form a subfamily of four heterodimers expressed only in leukocytes; αLβ2 (CD11a/CD18; LFA-1), αMβ2 (CD11b/CD18; Mac-1; CR3), αXβ2 (CD11c/CD18, p150,95, CR4), and αDβ2 (CD11d/CD18).

Leukocyte adhesion deficiency type I (LAD-I) is a rare autosomal recessive disorder arising from mutations in the gene of the integrin β2 subunit (Integrin β2, ITGB2), also known as the CD18 subunit of the CD11/CD18 leukocyte antigens, located in chromosome 21q22.3 (OMIM #116920). LAD-I patients suffer from recurrent bacterial or fungal infections, and exhibit marked increases in leukocyte number (leukocytosis), especially during periods of infection. Patients demonstrate a muted inflammatory response to infections as a result of poor leukocyte adhesion to the vascular wall and subsequent migration into the infected tissues. Early work led to the description of the deficiency as primarily due to the absence or highly diminished level of expression of the CD11/CD18 antigens [6–8]. Later, a diverse range of molecular defects leading to LAD-I have been characterized, including the expression of functionally defective CD11/CD18 antigens [9,10], and the expression of partially active integrins [11]. In this study, we have characterized two novel ITGB2 mutations in a LAD-I patient, one of which contains a 3′ splice-site mutation, and the other a deletion of 27,703 bp spanning the ITGB2 and PTTG1IP genes.

2. Materials and methods

2.1. Leukocytes and flow cytometry analysis

Data of flow cytometric analyses were collected using the FACSCaliber flow cytometer and processed with the CellQuest Pro software (Becton Dickinson). The mAb IB4 [12] was purchased from ATCC.

2.2. Genomic DNA and cDNA extraction

Blood (10 ml) were drawn from the patient, her parents, and an unrelated healthy individual. Genomic DNA was extracted using

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* Corresponding author. Address: School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551, Singapore. Fax: +65 67913856.
E-mail address: alaw@ntu.edu.sg (S.K. Alex Law).

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the QIAamp DNA Mini Kit (QIAGEN) according to the manufacturer's instructions. Total RNA was prepared with TRIZOL® Reagent (Invitrogen) and first strand cDNA synthesized using random primers (Roche Diagnostics), MuLV Reverse Transcriptase containing RNase Inhibitor (Applied Biosystems). Genomic DNA and cDNA were sent from Austria to Singapore for molecular analyses.

2.3. PCR, gel electrophoresis, DNA purification and DNA sequencing

PfuTurbo (Stratagene) was used for PCR with an initial denaturation at 95 °C for 2 min, and 30 cycles of denaturation at 95 °C for 1 min, annealing of primers at 5 °C below the T_m (supplied by 1st Base) for 30 s and extension at 72 °C for 1 min/kb followed by a final extension step at 72 °C for 10 min. Specific DNA products were excised for gel purification using a gel purification kit (Promega) according to the manufacturer's recommendations. Ten microlitres of purified plasmid DNA (100 ng/μl) or purified PCR product (30 ng/μl) were used as sequencing templates. The reactions were processed by AIT biotech (Singapore) or 1st Base (Singapore). Primers for PCR and sequencing reactions were synthesized at the 50 nmol scale (1st Base).

2.4. Inverse PCR

Genomic DNA (1 μg) was digested for 7 h at 37 °C with 25 U of *Eco*RI (NEB), and the enzyme was inactivated by heating at 65 °C for 20 min. After purification (PCR Cleanup Kit, Qiagen), digested DNA samples were ligated (circularized) at 4 °C for 18 h using 4000 U of T4 DNA ligase (NEB) in a 200 μl reaction. DNA was precipitated by the addition of 2 μl of 3 M sodium acetate, and 200 μl of isopropanol, with a total of 440 μg of glycogen as carrier. After washing twice in 70% ethanol, the DNA was resuspended in 20 μl of water. Circularized genomic DNA was amplified with relevant PCR primers.

3. Results

3.1. Patient

The patient is a Caucasian female born in 1996. Her parents are not known to be related. There was no significant delay in umbilical cord separation, which is usually the first sign of LAD-1 incidence [13]. She developed normally until an episode of a severe facial soft tissue and skin infection which required surgical intervention in 1999. A non-healing ulcer on her right arm was treated in 2002, while intensive periodontitis and gingivitis were also observed. When examined for leukocytosis, the patient exhibited an elevated white blood cell (WBC) count of 25,000 WBC/μl. Flow cytometric analyses of CD18 expression on patient granulocytes, monocytes and lymphocytes showed less than 2-fold over background, compared to over 35-fold in a normal control (Supplementary Fig. S1). The parents, confirmed later as carriers of the LAD-1 mutations, showed normal levels of CD18 expression, consistent with earlier reports on parents of LAD-1 patients [14–16]. Analyses were also performed for the CD11 antigens and results corroborated with an LAD-1 diagnosis (data not shown).

3.2. Genomic DNA sequencing of ITGB2 identified two novel intronic point mutations

The 16 exons of ITGB2 from the patient were amplified including approximately 50 bp of flanking sequences using PCR. Seven variations, different from the reference ITGB2 genomic sequence (RefSeqGene NG_007270.2), were identified and are presented in Table 1. Three were polymorphic codons, for Leu^{8} in the leader peptide, and Val^{367} and Val^{441}, which were reported previously [17]. The remaining variations were intronic, and included two previously reported variations, −11 in intron 5 and −47 in intron 8 [18,19] and two novel variations at position −10 in intron 2 (10 nucleotides upstream of the intron 2/exon 3 boundary) and position −29 in intron 5. In all seven cases, both wildtype and variant nucleotides were present.

3.3. The C to A point mutation in intron 2 resulted in a splicing defect

Two types of cDNA were found from the patient across the exon 2/3 junction. One is normal whereas the other has an insertion of a 43 bp of the 3′ end of intron 2: r.[59-43_59-1ins;59-10C>A] (Fig. 1A). The inserted sequence includes the C to A mutation found at the −10 position: g.23457C>A. The splice aberration would result in a premature stop codon (p.C19_V20ins11X12). In addition, the normal and mutant alleles are associated with the CTT and CTG codons for Leu^{8}, respectively.

In order to determine if the C to A mutation at position −10 of intron 2 is the cause for the aberrant splicing, the wildtype (CD18 I2-wt) and mutant (CD18 I2-mut) intron 2 were introduced into a CD18 cDNA expression plasmid and transfected into HEK293 cells. cDNA was prepared from mRNA and analysed (Fig. 1B). Although the unspliced intron was the major product species, weak bands corresponding to spliced cDNA were observed. Upon gel purification and re-amplification, the expected products (∼240 bp and ∼200 bp) were obtained (Fig. 1C).

Each CD18 I2 plasmid was co-transfected with CD11a, b, or c expression plasmids into HEK293 cells. Expression of the three CD11/CD18 integrins were detected by flow cytometry using the heterodimer specific mAb IB4 [12] on transfectants with the

Table 1
Summary of variants detected in the ITGB2 (CD18) gene of the LAD-1 patient.

Exon	Amino acid[a] affected	Codon in reference database[b]	Codon in patient	Mutation nomenclature[c]
Exonic variants				
2	Leu^{8}	CTG	CTG / CTT	g.23080G>T
10	Val^{367}	GTC	GTC / GTA	g.40312C>A
11	Val^{441}	GTT	GTT / GTC	g.41941T>C
Intron	Position	Nucleotide in reference database	Nucleotide in patient	Mutation nomenclature[c]
Intronic variants				
2	−10	C	C/A	g.23457C>A
5	−11	G	G/T	g.32095G>T
5	−29	C	C/T	g.32077C>T
8	−47	G	G/A	g.38732G>A

[a] The amino acid position given is according to the starting methionine (numbered "1").
[b] The reference database for ITGB2 is RefSeqGene NG_007270.2.
[c] The mutation nomenclature used was as described in Ref. [21].