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Mutation characterization and heterodimer analysis of patients with leukocyte adhesion deficiency: Including one novel mutation

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ABSTRACT

Background and aim: Leukocyte adhesion deficiency type 1 (LAD-I) is a rare, autosomal recessive disorder of neutrophil migration, characterized by severe, recurrent bacterial infections, inadequate pus formation and impaired wound healing. The *ITGB2* gene encodes the β2 integrin subunit (CD18) of the leukocyte adhesion cell molecules, and mutations in this gene cause LAD-I. The aim of the current study was to investigate the mutations in patients diagnosed with LAD-I and functional studies of the impact of two previously reported and a novel mutation on the expression of the CD18/CD11a heterodimer.

Materials and methods: Blood samples were taken from three patients who had signed the consent form. Genomic DNA was extracted and *ITGB2* exons and flanking intronic regions were amplified by polymerase chain reaction. Mutation screening was performed after Sanger sequencing of PCR products. For functional studies, COS-7 cells were co-transfected with an expression vector containing cDNA encoding mutant CD18 proteins and normal CD11a. Flow cytometry analysis of CD18/CD11a expression was assessed by dimer-specific IB4 monoclonal antibody.

Results: Two previously reported mutations and one novel mutation,p. Cys562Tyr, were found. All mutations reduced CD18/CD11 heterodimer expression.

Conclusion: Our strategy recognized the p.Cys562Tyr mutation as a pathogenic alteration that does not support CD18 heterodimer formation. Therefore, it can be put into a panel of carrier and prenatal diagnosis programs.

1. Introduction

Integrins are transmembrane receptors that are involved in cell–cell and cell-extracellular matrix. Interactions, immunity, wound healing, hemostasis and the development throughout the body. These proteins are large, heterodimeric cell adhesion molecules composed of α and β subunits. The β2 integrins (CD18) are β subunits in a family of heteromeric proteins: αLβ2 (LFA-1, CD11a/CD18), αMβ2 (Mac-1 or CR3, CD11b/CD18), αXβ2 (p150,95, CD11c/CD18) and αDβ2 (CR4, CD11d/CD18). These four proteins are expressed on leukocytes, except for αDβ2 (CR4, CD11d/CD18), which is only expressed on macrophages. The integrin β2 family has a crucial role in the immune system, because they recruit and activate leukocytes during inflammation [1–6]. Integrins can bind to extracellular matrix (ECM) glycoproteins, including collagens, fibronectins, laminins, and cellular receptors such as vascular cell adhesion molecule-1 (VCAM-1) and the intercellular cell adhesion molecule (ICAM) family [7] .Genetic alterations in the beta2-integrin gene play an important role in the pathophysiology of several diseases and genetic syndromes, including Leukocyte Adhesion Deficiency (LAD-I) and Systemic Lupus Erythematosus (SLE) [3]

CD18 is encoded by a gene located on chromosome 21q22.3 known as *ITGB2*. CD18 deficiency leads to incomplete formation and/or dysfunction of β2 integrins. More than 100 mutations have been reported in the *ITGB2* gene, including missense mutations (40%), splice

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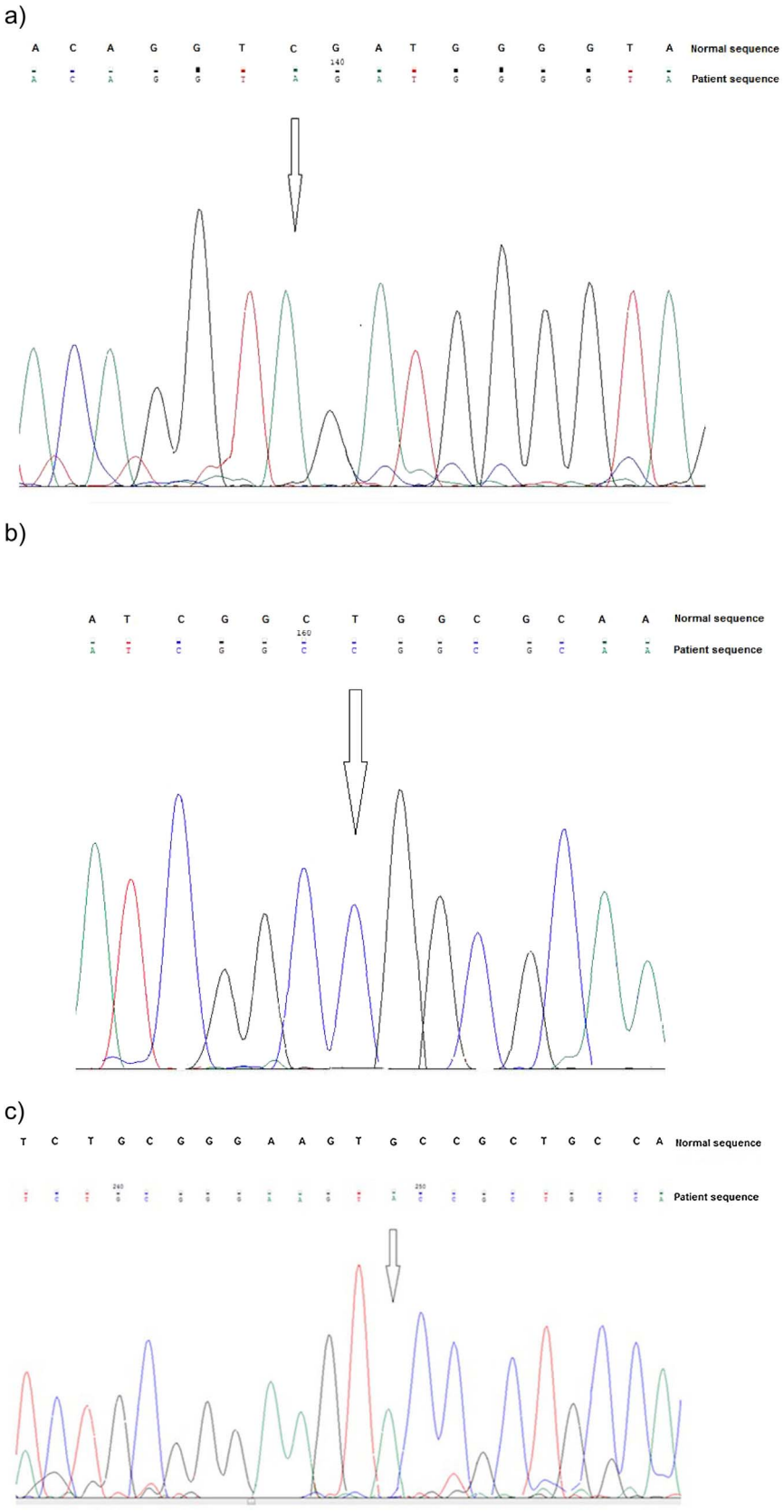

Fig. 1. Electropherogram of mutations.a) c.382 C > A on (−) strand leading to; p. Asp128Tyr b) c.754 T > C on (+) strand leading to; p.Trp282 Arg, and finally c) c.1885 G > A on (+) strand leading to; p. Cys563Tyr.Normal sequence is shown above each panel.