A point mutation c.473A > G of ITGB3 is responsible for the formation of the Wo[a] human platelet alloantigen

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CASE REPORT

A 36-year-old female (Wo) delivered a full-term girl in the 40th week of gestation with no signs of bleeding. Her neonatal platelet count was 29 G/L (dropping to 20 G/L), and fetal-neonatal alloimmune thrombocytopenia (FNAIT) was suspected. In the serological work-up, no anti-platelet antibodies were detectable using PAK Lx (Immucor Medizinische Diagnostik). Genetic testing did not reveal incompatibility for HPA-1, -2, -3, -4, -5, -6, -9 and -15. Two years later, Mrs. Wo gave birth to a white European full-term boy in the 39th week of gestation, again with no signs of bleeding. The neonatal platelet count was 31 G/L. Platelet antibody testing in the monoclonal antibody of platelet specific antigens (MAIPA) assay using random donor platelets was negative. In contrast, a MAIPA cross-match analysis between maternal serum and paternal platelets showed positive reactions with αIIbβ3, indicating an alloimmunization against a low-frequency antigen. On Day 1, the newborn was transfused with random donor platelets. The platelet count rose to 170 G/L, remained above 50 G/L without further transfusions, and was in normal range at Day 28.

METHODS

Full-length sequencing of *ITGA2B* and *ITGB3* was carried out as described previously.[1] Briefly, coding regions of paternal genomic DNA were PCR amplified with primers corresponding to intronic sequence surrounding all exons. PCR was carried out using a Fast-Start High Fidelity PCR system (Roche Diagnostic Corp.). Automated sequence analysis was performed in both directions on a genetic analyzer (ABI 3100, Applied Biosystems). Nucleotide sequences of PCR primers, sequencing, and reaction conditions are available upon request.

Full-length β3 cDNA encoding for the Wo[a] variant was produced by site-directed mutagenesis using the Quick Change Mutagenesis Kit (Stratagene) as previously described.[2] Allele specific constructs encoding wild-type β3 (Gln_{132}) or mutant β3 (Arg_{132}) were transfected into HEK 293F cells, together with an αIIb construct. Surface expression of αIIbβ3 was measured by flow cytometry (FACS Canto II, Becton Dickinson), as outlined previously.[2] To confirm that p.Gln132Arg is responsible for the formation of the Wo[a] epitope, 3 x 10^5 transfected cells per well were used in a modified MAIPA.[3]

RESULTS

Sequencing *ITGB3* from paternal DNA showed the presence of a nucleotide substitution c.473A > G located in exon 4 of ITGB3 (Fig. 1A). This mutation predicts Gln (CAG) at position 132 in Wo[a]-negative and Arg (CGG) in Wo[a]-positive individuals (p.Gln132Arg). The mutation was deposited in GenBank (accession number MN624129). No sequence variation was detected in *ITGA2B*. The *ITGB3* c.473A > G mutation was also detectable in heterozygous state when sequencing DNA from the newborn. In a glycoprotein specific assay (Fig. 1B), HEK 293F cells expressing αIIb in complex with either wild-type (Gln_{132}) or mutant (Arg_{132}) β3 were incubated with maternal serum, healthy control donor serum, or serum

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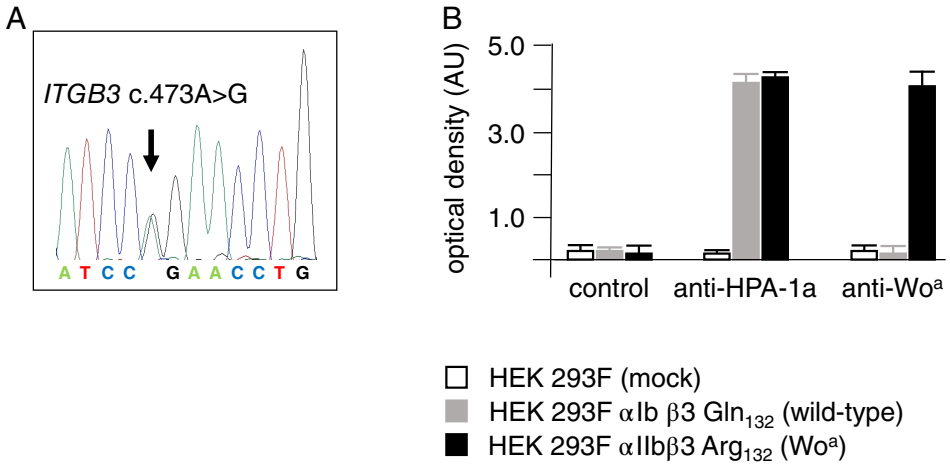

Fig. 1. (A) Nucleotide substitution c.473A > G located in exon 4 of ITGB3 as detected in paternal DNA (GenBank accession number MN624129, SNP database rs_748901429). (B) Serological confirmation of the Wo[a] epitope. HEK 293F cells were used after mock transfection (white), transfection with wild-type αIIbβ3 (gray) and transfection with αIIbβ3 after introduction of the c.473A > G mutation in ITGB3 (black) in a modified MAIPA assay. Transfected cells were incubated with control serum from a healthy blood donor, with serum containing anti-HPA-1a antibodies, or with the maternal serum containing anti-Wo[a], as indicated. After cell lysis, αIIbβ3 was immobilized to the microtiter plate using complex-specific monoclonal antibody Gi5. Presence of human IgG in the immobilized complex was detected using horseradish peroxidase-labeled goat-anti-human-IgG and substrate buffer. Optical density (OD) was read at 492 nm. Wild-type HEK293F αIIbβ3 Gln_{132} (gray) and mutant HEK293F αIIbβ3 Arg_{132} (black) were reactive with anti-HPA-1a (OD 4.19 ± 0.19 and 4.32 ± 0.16, respectively). In contrast, the wild-type form (gray) did not react with anti-Wo[a] (OD, 0.14 ± 0,19), whereas the mutant form (black) gave strong reactions (OD, 4.11 ± 0.47). All data given as mean ± standard deviation from n = 3 independent experiments.

containing anti-HPA-1a antibodies, demonstrating the specific recognition of p.Gln132Arg by anti-Wo[a].

ALLELE FREQUENCY

Summary data from the Exome Aggregation Consortium indicate allele frequencies of 0.99999 for ITGB3 c.473A and 0.00001 for ITGB3 c.473G.[4]

SUMMARY

We observed a case of FNAIT in a newborn with a platelet count of 31 G/L. Serological analysis of the maternal serum revealed an immunization against αIIbβ3 on paternal platelets only, indicating the presence of an antibody against a rare alloantigen. Sequencing analysis and studies with mutant transfected cells showed that ITGB3 c.473A > G (p.Gln132Arg) is responsible for the formation of a new antigenic determinant on β3, termed Wo[a]. The antibody was overlooked in the initial work-up of the elder sibling where no cross-match study was performed. This demonstrates once more that a serological cross-match between paternal platelets and maternal serum should always be performed whenever FNAIT is suspected.

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