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Mutation Report

G→A TRANSITION AT NUCLEOTIDE 2110 IN THE HUMAN PLATELET GLYCOPROTEIN (GP) IX GENE RESULTING IN ALA^{139}(ACC)→ THR(GCC) SUBSTITUTION

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The glycoprotein (GP) IX is a platelet membrane-associated protein and forms an 1 : 1 non-covalent complex with GP Ib, a heterodimeric transmembrane protein consisting of a disulfide linked 140 kD α chain and 22 kD β chain (Roth, 1991). This GPIb/IX complex serves as a receptor for plasma von Willebrand factor (vWF) to mediate platelet adhesion to the damaged vascular wall (Lopez, 1994). The GPIbα chain provides the vWF binding site, but the individual contribution of GPIX to adherence is unclear. GPIX is a relatively small (Mr 20,000) surface protein, and employed in the efficient membrane expression of a functional GPIb/IX complex (Lopez *et al.*, 1992). The gene of GPIX has been cloned and mapped to chromosome 3 (Hickey *et al.*, 1990; Hickey and Roth, 1993). Genetic analyses of the patients with Bernard-Soulier syndrome (BSS), a hereditary qualitative platelet disorder characterized by a quantitative and/or qualitative abnormality of the GPIb/IX complex, revealed that the abnormality of the GPIX gene was able to cause BSS (Wright *et al.*, 1993; Clemetson *et al.*, 1994). In the course of GPIX gene analysis, we found a novel variant of a G→A transition at nucleotide 2110 resulting in Ala^{139}(GCC)→Thr(ACC) replacement. Relationship between this variant and BSS phenotype was also studied in this report.

Polymerase chain reaction (PCR) was performed to amplify the region of the

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GPIX gene encompassing the variant site. The primers used for PCR were as follows:

GPIXLi-9; 5′-TCACCTATCTGCGCCTCTGGCTGGAG-3′,

GPIXLi-10; 5′-GCTGACCAGGCTTGGTGGAGTCTGGG-3′,

which corresponds to nucleotide 1925–1950 for GPIXLi-9 and 2232–2257 for GPIXLi-10 (GenBank HUMGPIX, M80478). The PCR amplification was performed by a DNA thermal cycler (Perkin Elmer Cetus, Norwalk, CT) with 2.5 U of *Taq* DNA polymerase (Takara, Kyoto) in a total volume of 100 μl of standard PCR buffer containing 500 ng of the genomic DNA, 50 pmol of each primer and 10% (v/v) dimethyl sulfoxide. After subcloning the amplified fragments into the pGEM-T vectors (Promega, Madison, WI), nucleotide sequencing was performed using Deaza G/A T7 Sequencing Kits (Pharmacia, Uppsala, Sweden) with a primer complementary to the vector or the insert sequence. Figure 1A showed a variant allele sequence with a G→A transition at nucleotide 2110 of the GPIX gene. Notes that this transition creates a new *Bst*EII site on the PCR fragment (Fig. 1B, upper).

An allele-specific restriction enzyme analysis was performed to detect this variant in Japanese. Genomic DNAs from unrelated 53 healthy individuals were amplified by PCR using above mentioned primers. The obtained PCR fragments

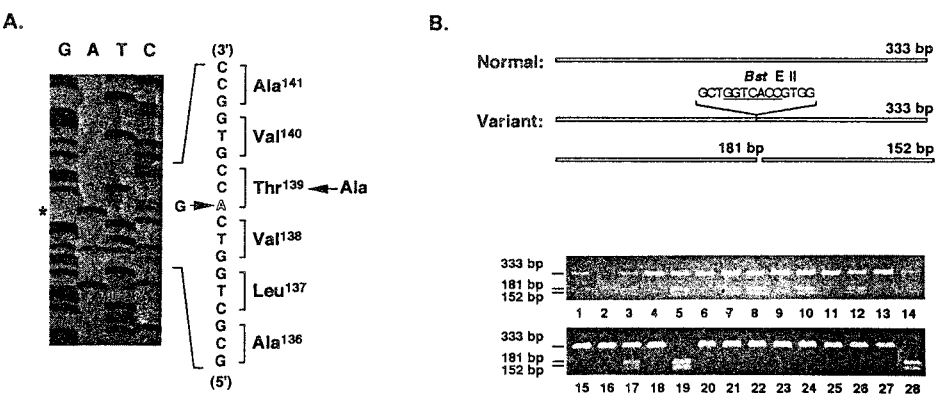

Fig. 1. A. Autoradiograph illustrating a variant DNA sequence. The observed variant sequence is shown. G→A transition at nucleotide 2110 results in Ala^{139}(GCC) →Thr(ACC) substitution. An asterisk denotes a variant band. B. Allele-specific restriction analysis of the PCR products. (Upper) Schema of *Bst*EII digestion of the PCR fragments. G→A transition at nucleotide 2110 creates a new *Bst*EII restriction site (underlined). After treatment of the PCR fragments with *Bst*EII, a variant allele produces 181-bp and 152-bp fragments. (Lower) The amplified 333-bp PCR fragments were digested with *Bst*EII, and analyzed by electrophoresis through a 2.5% agarose gel. Patterns of DNA digests from 26 individuals are shown. Corresponding molecular sizes are shown at left side. Lane 1: not digested. Lanes 3–28: digested with *Bst*EII.

were digested with an endonuclease *Bst*EII (Takara, Kyoto) and analyzed by electrophoresis through a 2.5% agarose gel (Fig. 1B, lower). Out of 53 individuals examined, 30 were homozygous for G/G and 19 were heterozygous for G/A. Only 4 individuals were homozygous for A/A. The allelic frequency, estimated from 53 unrelated Japanese, is as follows: for the 2110 G/A variation in the GPIX gene, G=0.75 and A=0.25.

This G→A transition results in Ala^{139}→Thr substitution. Both Ala and Thr have aliphatic side chains, but the hydroxyl group of Thr side chain makes it more hydrophilic. Since Ala resides within a transmembrane domain of the GPIX polypeptide (Hickey *et al.*, 1989), Ala^{139}→Thr substitution may result in an insufficient surface expression of the GPIb/IX complex. Four individuals homozygous for A/A were analyzed their surface expression of the GPIb/IX complex by a fluorescence-activated cell sorter (FACS). Ristocetin-induced platelet agglutination test (RIPA) (Kinlough-Rathobone *et al.*, 1983) were also performed to analyze if the functional GPIb/IX complex occurred on the platelet surface. Both FACS analysis and RIPA failed to show different patterns from normal (data not shown), therefore, the G→A transition described here is again confirmed as a *Bst*EII polymorphism of the GPIX gene with Ala^{139}→Thr substitution. This *Bst*EII polymorphism will be useful for analyzing the GPIX gene or for a linkage analysis as an intragenic marker.

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