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RFLP Report

*Sty*I POLYMORPHISM AT NUCLEOTIDE 1610 IN THE HUMAN PLATELET GLYCOPROTEIN Ib ALPHA GENE

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The glycoprotein (GP) Ib alpha is a platelet membrane-associated protein, and forms the GPIb/IX hetero-complex, a receptor for plasma von Willebrand factor (Roth, 1991). Current understanding suggests that this receptor-ligand interaction occurs in a condition of high shear rate and plays a critical role in the initial step of hemostasis (Ikeda *et al.*, 1991; Weiss *et al.*, 1989). The gene of GPIb alpha has been cloned (Wenger *et al.*, 1988), and mapped to chromosome 17p12-p13 (Wenger *et al.*, 1989). Extensive investigations have revealed the genetic abnormalities of the GPIb alpha gene, and some of them are responsible for the pathogenesis of Bernard-Soulier syndrome. Other mutations such as *Taq*I and *Bsu*36I polymorphisms (Petersen and Handin, 1992), methionine^{145}/threonine amino acid dimorphism (Murata *et al.*, 1992; Kuijers *et al.*, 1992) and molecular weight polymorphism (Moroi *et al.*, 1984) have also been reported. When analyzed the GPIb alpha gene of patients with Bernard-Soulier syndrome, we found a novel polymorphism in the GPIb alpha gene. The sequence of this substitution and its allelic frequency in Japanese were shown in this report.

We carried out polymerase chain reaction (PCR) to amplify the GPIb alpha gene with its adjacent 5′- and 3′-flanking regions. The oligonucleotide primers were synthesized according to the report by Simsek *et al.* (1994). The PCR amplification was performed by a DNA thermal cycler (Perkin Elmer Cetus, Normark, CT) with 2.5 U of Taq DNA polymerase (Takara, Kyoto, Japan) in a total volume of 50 μl containing 500 ng of the genomic DNA and 50 pmol of each

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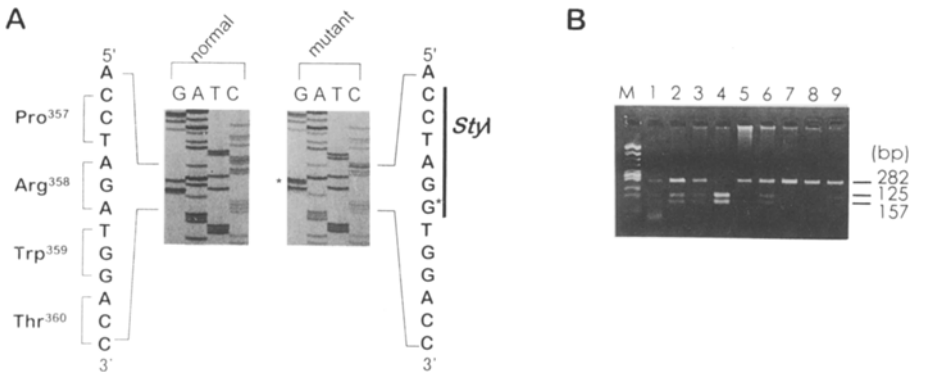

Fig. 1. A. Autoradiograph illustrating a normal and a variant DNA sequences. Left panel shows a normal allele sequence, and right panel shows a variant allele with G at nucleotide position 1610. An asterisk denotes a variant band. Notes that A→G substitution creates a new *Sty*I restriction site in the GPIb alpha gene. B. Allele-specific restriction analysis of the healthy volunteers. The amplified 282-bp fragments were digested with *Sty*I, and analyzed by electrophoresis through a 2.5% agarose gel. Patterns of DNA digests from nine individuals are shown. Lanes 5, 7, 8 for A/A. Lanes 1, 2, 3, 6, 9 for A/G. Lane 4 for G/G. M for marker lane (ϕX174/*Hin*cII digestion).

primer mentioned above. The amplified fragments were subcloned into M13-based vectors for DNA sequence. 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. The amplified fragment had an A→G substitution in a coding region of the GPIb alpha gene at nucleotide 1610 (Arg^{358} (AGA)→Arg(AGG*) of the published sequence (Wenger *et al.*, 1988), resulting in creation of a new *Sty*I restriction site (Fig. 1A).

An allele-specific restriction enzyme analysis was performed to detect this substitution in Japanese. Following primers were prepared to amplify the DNA sequence covering a mutation site: StyI-1, 5′-GGTGCGTGCCACAAGGACTGT-3′; StyI-2, 5′-TTTGGGGCGGGCTCCGGGACG-3′, which correspond to nucleotides 1448–1468 for StyI-1 and 1729–1709 for StyI-2. Genomic DNAs were prepared from unrelated 33 healthy individuals, and then PCR was performed using these primers. The obtained PCR fragments were digested with an endonuclease *Sty*I (Nippon gene, Toyama, Japan) (Fig. 1B). Out of 33 individuals examined, 17 were homozygous for A/A and 14 were heterozygous for A/G. Only two individuals were homozygous for G/G. The allelic frequency, estimated from 33 unrelated Japanese, is as follows: for the 1610 A/G variation in the GPIb alpha, A=0.73 and G=0.27.

This A→G substitution does not alter an amino acid sequence. The platelet counts and ristocetin-induced platelet aggregation were also normal in people used in this study. This *Sty*I polymorphic site will be useful for analyzing the GPIb

alpha gene or a linkage analysis as an intragenic marker.

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