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Allele 14 of vWA is characterized by 3′-flanking nucleotide substitutions and a TTAT insertion

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Abstract

Intron 40 of the vWF gene is known to harbor three different STRs, which were separately reported by Kimpton et al. (vWA), by Peake et al. (vWF-P), and by Ploos van Amstel et al. (vWF-A). En bloc detection of the three STRs (F8VWF) and sequencing of the F8VWF alleles revealed novel base substitutions, tetranucleotide insertions, and tetranucleotide repeats. It is noted that the nucleotide substitutions and insertions were observed exclusively in those F8VWF alleles having the vWA allele 14.

Keywords: Sequence; Mutation; DNA polymorphism; STR; vWF

1. Introduction

Intron 40 of the vWF gene is known to harbor three different STRs, which were separately reported by Kimpton et al. (vWA), by Peake et al. (vWF-P), and by Ploos van Amstel et al. (vWF-A) [1–4]. Amplification of the variable segment spanning all three repeat regions (F8VWF) has been reported by Mercie et al. [5]. However, there are no sequence data on the whole F8VWF. In this study, we first report allele distributions and sequence data of the F8VWF region.

2. Materials and methods

Genomic DNA was recovered from peripheral leucocytes of 47 Japanese individuals by using DNA Extractor WB kit. Two primers, W31 (5′-GCCCTAGTGGATGATAAGAAT-

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AATCAG-3′) and W32 (5′-GCAGACTTCTACTGTTTTAGGTAAGTG-3′), were used to amplify the F8VWF rregion. PCR amplification was performed according to the published protocol with minor modifications [5]. The sequencing reaction was done by using the BigDye Terminator Cycle Sequencing FS Ready Reaction Kit.

3. Results and discussion

In this survey, a total of 13 different length alleles were identified for F8VWF in 47 Japanese individuals, ranging from 746 to 810 bp. The 13 representative alleles were sequenced to be used as an allelic ladder. We did not detect alleles of 750, 754, 758, and 794 bp in this study.

Finally, a total of 23 different length alleles were sequenced and analyzed. We found five nucleotide substitutions as follows: nt 1761 T→C, nt 1849 T→A, nt 2122 C→T, nt 2180 C→T, and nt 2192 C→T (Fig. 1). One TTAT tetranucleotide insertion was found occurring at nt 2057 and 2058 (Fig. 1). Polymorphisms in repeat numbers of four tetranucleotide repeats, TATC (nt 1982), ATCT (nt 2193), TGTA (nt 2234), and TCTA (nt 2258) were identified in the intron 40 (Fig. 1). These substitutions and insertion were observed only in vWA allele 14. Thus, F8VWF alleles were found to be classified into two types: alleles without (type 1) or with (type 2) allele 14 of vWA.

In conclusion, as for molecular evolution of vWA alleles, Wiegand et al. [6] suggested by comparing the repeat structure of allele 14 between humans and hominoid primates that allele 14 is an ancestral allele. However, exclusive occurrence of the five nucleotide substitutions and insertion in vWA allele 14 may give a reverse insight to the suggestion although the number of fully sequenced alleles has not been enough to draw a conclusion yet in this study. Downstream sequencing of various vWA alleles of hominoid primate samples would resolve the question.

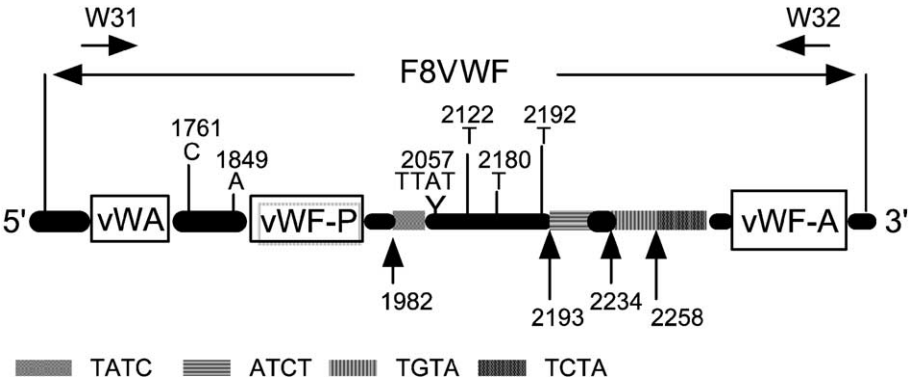

Fig. 1. Sequence structure of the polymorphic regions within intron 40 of the vWF gene. The positions of the four polymorphic repeats, newly detected nucleotide substitutions and the TTAT insertion are indicated by vertical arrows in relation to the sites of the three STRs. The primers used to amplify the F8VWF regions as a whole are shown at the top.

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