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Forensic STRs as potential disease markers: A study of VWA and von Willebrand's Disease

Rebecca Laird [a,*], Peter M. Schneider [b], Silvana Gaudieri [a,c]

[a] Centre for Forensic Science, University of Western Australia, Nedlands, Western Australia, Australia
[b] Institute of Legal Medicine, University of Cologne, Cologne, Germany
[c] School of Anatomy and Human Biology, University of Western Australia, Nedlands, Western Australia, Australia

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Abstract

In recent years it has been established that non-coding variants may be in linkage disequilibrium (LD) with coding variants up to several thousand base pairs away forming haplotype blocks. These non-coding markers may be haplotype specific and, therefore, informative regarding the surrounding coding sequence. In this study, we chose to study the VWA short tandem repeat (STR) as it is targeted in all major commercial kits utilized in routine forensic DNA profiling and is located in the von Willebrand Factor (vWF) gene; a gene associated with von Willebrand's Disease (vWD). We examined the VWA STR together with single nucleotide polymorphisms (SNPs) located throughout the vWF gene to identify haplotype structures and the extent of LD between markers in the region. Several areas exhibiting LD were identified by population data analysis in the 178 kilobase (178kb) vWF gene, which was supported by family studies. However, there appeared to be no evidence of LD blocks surrounding the VWA STR and evidence for recombination within 3 kb of VWA, hence, it is unlikely that VWA STR alleles could be used to predict haplotypes within the vWF gene that are associated with different forms of vWD.
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1. Introduction

In recent years several new concepts in genome structure have been established, which may impact on the selection of markers for forensic use. At present, short tandem repeats (STRs) are the predominant markers used in criminal stain typing as well as in relationship testing. STRs have been selected from non-coding regions of DNA, commonly termed ''junk DNA''. It has been accepted that selecting markers from such regions will not provide information about inherited genetic conditions or characteristics [1]. However, since the original selection of the STR panels for use in forensic biology it has been shown that the genome consists of large areas of sequence (up to several hundred kilobases (kb)) that exhibit high levels of linkage disequilibrium (LD) flanked by recombination 'hotspots' or LD breakdown [2–4]. These regions are termed genomic or haplotype blocks and can be conserved across different populations or may occur uniquely within particular population groups [5,6]. Recombination is observed between, but rarely within the genomic blocks, and thus markers within a block can provide information about a genetic polymorphism within the same block several kb away forming haplotypes. In other words, non-coding polymorphic markers, such as STRs, may provide information about nearby coding sequence. In this study, the STR VWA is used as a case study to explore whether the STR polymorphisms are informative for block haplotypes, or extended gene haplotypes, associated with disease.

Within the commonly used CODIS STRs, five are located within genes (Table S1). One example is VWA, present within intron 40 of the von Willebrand Factor (vWF) gene; a gene associated with von Willebrand's Disease (vWD). VWF is a glycoprotein present in blood and is known to promote platelet adhesion and aggregation, and act as a carrier for the plasma procoagulant co-enzyme, factor VIII [7,8]. The duplication of functional regions within the vWF gene and the presence of entire duplicated segments in the vWF pseudogene, supports the notion that areas of the vWF gene are evolving *en-block* and

* Corresponding author. Tel.: +61 8 6488 7286; fax: +61 8 6488 7285.
E-mail address: r.laird@murdoch.edu.au (R. Laird).

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that the gene is likely to exhibit areas of LD which could be marked by haplotype tags [9].

VWD is a heterogeneous bleeding disorder characterized by a variety of quantitative (type 1) and qualitative (type 2) abnormalities of vWF, or a total deficiency of the protein (type 3) [10]. Mutations causing vWD can result in impairment of the assembly, intracellular targeting, or secretion of vWF multimers, impair the survival of vWF in plasma, or the function of specific ligand binding sites [11,12]. Type 1 vWD (60–80% of vWD cases) may be caused by mutations throughout the entire protein, which overlap with those identified in vWD type 3 [12]. For type 2 vWD (20–30% of vWD cases) four subtypes exist: 2A, 2B, 2M and 2N. VWD type 2A is caused by single amino acid substitutions within the repeated A2 domain of the mature vWF subunit, encoded by exon 28 (Fig. 1(A)). VWD Type 2B is usually caused by substitutions clustered in a discrete region on the A1 domain and appears to mark the location of a regulatory site that normally inhibits the binding of A1 to platelet glycoprotein GPIb. In contrast, type 2M is caused by decreased or absent binding of A1 to GPIb on the platelets. So far, the few known vWD type 2M mutations are located in the A1 domain and disrupt binding to platelet GPIb. Type 2N is characterized by lower factor VIII and is usually caused by changes in the D' and D3 domains encoded by exons 18–28 [8,11] (Fig. 1(A)).

In this study, SNPs within the vWF gene in population and family samples were examined to determine possible LD blocks and recombination hotspots within the gene. The VWA STR is also analyzed in the context of extended gene haplotypes to determine whether the marker may be informative regarding genetic changes relevant to vWD.

2. Materials and methods

2.1. DNA samples

Taiwanese population samples ($n = 50$) were kindly donated by Dr. C.E. Pu from the Scientific and Technical Research Centre, Ministry Justice Investigation Bureau, Taipei, Taiwan. CEPH family DNA was purchased from the NIGMS Human Genetic Cell Repository, Coriell Institute of Medical Research.

2.2. VWA STR typing

The VWA STR was typed for each of the DNA samples using the AmpF*l*STR® SGM plus® kit (Applied Biosystems) according to the manufacturer's protocol. For each sample, 1.5 ng of DNA was added to a PCR reaction containing 21 μL of AmpF*l*STR PCR reaction mix, 1 μL of AmpliTaq Gold DNA Polymerase and 11 μL of AmpF*l*STR SGM Plus primer set. The reaction was run on a GeneAmp® 2400 with the cycling conditions; 95 °C for 11 min, followed by 28 cycles of 94 °C for 1 min, 59 °C for 1 min and 72 °C for 1 min, and finally, a

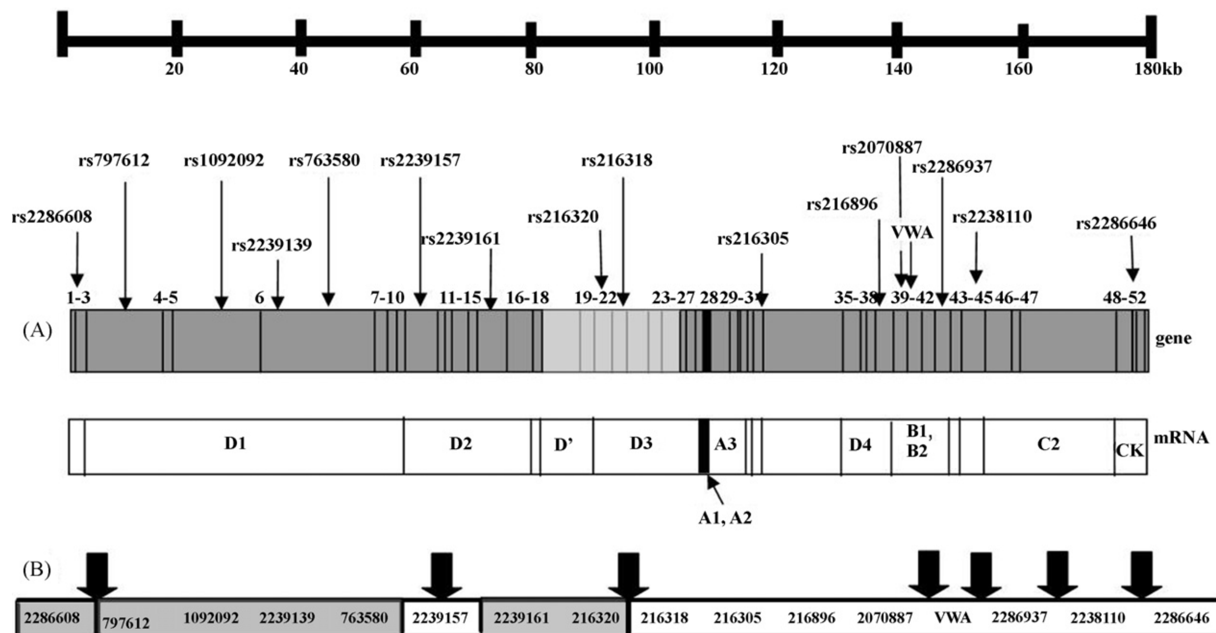

Fig. 1. vWF gene with SNP locations and predicted LD structure. (A) The 178 kb vWF gene is shown as the upper shaded box with each vertical bar representing an exon and numbered. The location of the selected SNPs are indicated as well as the VWA STR located in intron 40. The second bar (not shaded) denotes the functional areas of the 8.7 kb vWF mRNA, with each domain directly below the exons which encode it (not to scale). Exon 28 is associated with types 2A, 2B and 2M vWD and encodes the A1 and A2 domains. The region implicated with type 2N vWD is shaded light grey and includes exons 18–25 (top bar). Types 1 and 3 vWD have been correlated with mutations throughout the gene. SNPs were selected to cover the entire vWF gene with an average heterozygosity between 0.3 and 0.5 (wherever possible) as recorded in the NCBI SNP database (http://www.ncbi.nlm.nih.gov/SNP). The typed SNPs are indicated with arrows. (B) The horizontal bar denotes each SNP typed with the SNaPshot technique for the population and CEPH family samples. The bold arrows indicate minimal points of recombination between SNPs as predicted (numbers = rs SNP numbers) from the three generation families and the vertical lines between SNPs mark possible block boundaries in the vWF gene as indicated by the LD analysis of the population data. The shaded areas mark the location of the three possible LD blocks.

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