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Research article

Analysis strategies to establish vWF intron 40 haplotypes

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Abstract

Sequencing of a 0.65 kb region in the intron 40 of the vWF gene demonstrated a complex variability. Five STRs named Pol K, F, P1, P2-a and P2-b and an indel polymorphisms (I) are present. We established a routine analysing method to puzzle out the Pol K/F/I/P haplotypes which does not require a sequencing procedure. To recognise the combined polymorphisms as haplotypes, we performed short and middle range PCRs in combination with Nde I and BsmA I restriction tests. Comparison of the amplicon and restriction fragment length reveals the most likely haplotypes of each person involved a kinship test. Furthermore, a SNP allele specific PCR was employed. Additional information can be achieved by typing Pol P2-a and P2-b. Establishing of intron 40 vWF haplotypes using the methods described here can greatly support the resolution of complex kinship cases. This statement is illustrated by demonstration of a family study.

Keywords: vWF intron 40; Kinship testing; Haplotyping

1. Introduction

Intron 40 of the von Willebrand factor (vWF) exhibits several microsatellite polymorphisms [1–5]. One of this STRs is the well known CODIS marker. Recently, we described the complex variability examined by sequencing analysis of a 0.65 kb region of the intron 40. We demonstrated five STRs which we named Pol K, F, P1, P2-a and P2-b (Fig. 1). Furthermore, two indel polymorphisms and five SNPs were found and could be arranged into a system of three haplotypes (a–c). SNP haplotype c is associated with Pol K allele 14 and shows an atypical repeat structure [6]. This study was performed to establish a method for haplotyping based on PCR and restriction fragment analysis.

2. Methods

DNA specimen with known vWF Pol K alleles were taken from routine kinship cases. Primers were electronically designed (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) (Table 1).

PCR (25 μl): 0.1–1 ng DNA, 200 μM each dNTP, 1.5 mM $MgCl_2$, 0.5 μM of each primer, 1 U Taq polymerase (Applied Biosystems); 95 °C, 3 min soak; 94 °C, 30 s; 50/58 °C, 1 min (PTC-200 cycler; MJ Research, USA).

The two-side-labelled long amplicons (A) involving all polymorphisms from K to P were restricted for at least 4 h at with BsmA I to separate the Pol K alleles from the summarized Pol F, I and P alleles, and with Nde I (NEB, Ipswich, USA) to separate the summarized Pol K, F and I alleles from the Pol P alleles, respectively. The resulting PCR products were resolved and detected by capillary electrophoresis in the denaturing polymers POP4 (PerkinElmer) in the ABI 310 sequencer (PerkinElmer) following standard protocols. Amplicon sizing was supported using the Internal Lane Standard 600 (Promega). Calibration of the allelic ladder was done using sequenced samples [6]. Haplotype reconstruction was done by comparing the long amplicons (summarized Pol K, F, I and P alleles) with the restricted fragments and the short amplicons.

3. Results and discussion

Table 2 demonstrates the procedure to puzzle out the vWF Pol K/F/I/P haplotype. The haplotypes of each person can be determined by comparing the length of all amplicons and all

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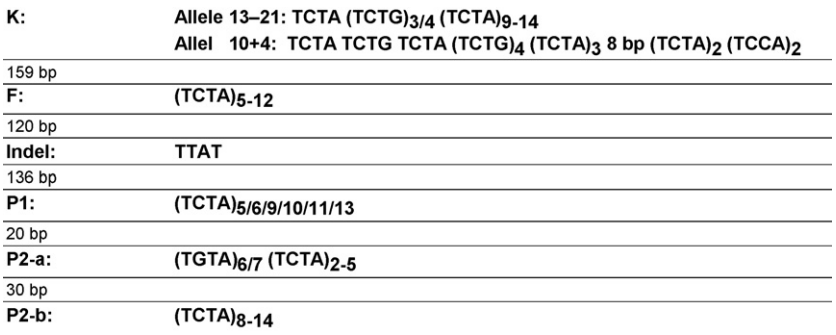

Fig. 1. The intron 40 of the vWF gene contains five juxtaposed STRs named K, F, P1, P2-a, P2b and an TTAT/- indel polymorphism.

Table 1
Primers and annealing temperatures

Amplicon	Pol	Primer name	Primer sequence	Annealing (°C)
A	K + F + I + P	VWA-FAM VWA-HEX	5′-FAM-AAAGCCCTAGTGGATGATAAGAA 5′-HEX-TGATGATGGAGACAGAGATTACA	58
B	F[a]	F-FAM F-rev.	5-FAM-CCTATCTCTATCTAAGCTACATA 5′-GTGGTTAGATAGATTAGACAGAC	54
C	SNP a/b F + I + P	SNP a-FAM + SNP b-HEX SNP rev.	5′-FAM-TATCCTGTCTCTATCTATCCT**A**TG 5′-HEX-TATCCTGTCTCTATCTATCCT**T**TG 5′-TGG AGA CAG AGA TTA CAT GG	58
D	P2	P2 VWA-HEX	5′-TGTACCTAGTTATCTATCCTGTATG 5′-HEX-TGATGATGGAGACAGAGATTACA	58
E	P2-b[a]	P2b FAM P2b rev.	5′-FAM-TCTATCAAATCTATCTCATGTATCT 5′-AAGTGATGATGATGGAGACA	54

[a] Duplex PCR possible.

Table 2
Example for the haplotype reconstruction including SNP type in a family with two children (without P subtypes)

Person	K	F	K + F + I	I	F + I + P (SNP)	P	Haplotype: SNP/K/F/I/P
Mother	17/17	6/12	23/29	0	31a/36b	24/25	a/17/6/0/25–b/17/12/0/24
Child 1	16/17	5/6	21/23	0	27a/31a	22/25	a/16/5/0/22–a/17/6/0/25
Child 2	14/17	6/6	20/23	0	30b/31a	24/25	b/14/6/0/24–a/17/6/0/25
Father	14/16	5/6	20/21	0	27a/30b	22/24	b/14/6/0/24–a/16/5/0/22

restriction fragments. In addition, SNP-allele-specific PCR resulted in amplicons type C hosting Pol F, I and P. This corresponds to the HEX labelled BsmA I restriction fragments produced by cleaving of amplicon A. Table 3 shows additional polymorphic information for this family with P subtyping. Typical haplotypes were known from the sequencing study carried out before [6]. This information was assistant to reconstruct the most likely allele combinations.

Estimation of vWF haplotypes including the Pol K, F, Indel, P and SNP type makes available a high number of low frequency intron 40 haplotypes, which provide an enormous potential for kinship analysis. If the vWF Pol K alleles are known from routine casework or multiplexes, three further PCR reactions and one to two restriction analyses have to be done. Pol P subtyping can increase the information, but correct haplotype reconstruction is not possible in all cases.

Table 3
Reconstruction of the most likely Pol P subtypes for this family (unlikely allele combinations are in brackets)

Person	Pol P	Pol P2	Haplotype SNP/P1/P2	Pol P2-b	Haplotype SNP/P2-a/P2-b
Mother	b24/a25	19/20	b/5/19 (4/20)–a/5/20 (6/19)	11/11	b/8/11–a/9/11
Child 1	a22/a25	17/20	a/5/17 (2/20)–a/5/20 (8/17)	8/11	a/9/8 (6/11)–a/9/11 (12/8)
Child 2	b24/a25	18/20	b/6/18 (4/20)–a/5/20 (7/18)	10/11	b/8/10 (7/11)–a/9/11 (10/10)
Father	a22/b24	17/18	a/5/17 (4/18)–b/6/18 (7/17)	8/10	a/9/8 (7/10)–b/8/10 (10/8)

4. Conclusions

We established a routine analysing method to puzzle out the Pol K/F/I/P haplotypes, which does not require a sequencing procedure. To recognise the combined polymorphisms as haplotypes we performed short and middle range PCRs in combination with Nde I and BsmA I restriction tests. Comparison of the amplicon and restriction fragment length reveals the most likely haplotypes of each person involved a kinship test. Furthermore, a SNP type specific PCR was employed. Additional information can be achieved by typing Pol P2-a and P2-b. Establishing of intron 40 vWF haplotypes using the methods described here can greatly support the resolution of complex kinship cases.

Conflict of interest

None.

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