Abstract: P1622

Title: GENE CONVERSION CASES FOUND DURING THE VON WILLEBRAND GENE STUDY

Abstract Type: e-Poster Presentation

Session Title: Bleeding disorders (congenital and acquired)

Background:

Gene conversion between the von Willebrand gene (*VWF*) on chromosome 12 and its pseudogene on chromosome 22 is a common phenomenon. It usually occurs in exon 28 of *vWF* coding A1 domain, which plays a role in binding with glycoprotein (GP)Ibα on a platelet membrane.

Aims:

Our aim was to find cases of gene conversion and to estimate the frequency of this event for our patients. We also tried to connect this particular gene defect with patient phenotype and examine conversed pseudogene to reconstruct the conversion mechanism.

Methods:

Patients with suspected von Willebrand disease (vWD) diagnosis were included in the study (N=64). Total DNA was extracted from the whole blood by the phenol-chloroform method. PCR of the exon 28 of the *vWF* gene and for the homologous region of pseudogene was performed using primers developed in our laboratory. The sequences were aligned with Genbank references for the *VWF* gene (NG_009072.1) and the *VWFP1* pseudogene (M60676.1).

Results:

We have sequenced the 28th exon of the *VWF* gene for 64 patients. In two cases (patients A and B) substitutions that are characteristic for pseudogene sequence were found. Patient A had three substitutions in a heterozygous state in the known hot spot of gene conversion in the *VWF* gene: two were nonsynonymous (p.Pro1266Leu, p.Val1279Ile) and one synonymous (c.3789G>A). Pedigree analysis of the patients' A family showed that all the three substitutions are located on the same allele and patients' mother had the same substitutions (and vWD); patient`s A son had not got the substitutions and no vWD simptoms. Patient B had two heterozygous changes in known gene conversion area, but both of them were polymorphisms (c.3686T>G and c.3692A>C); pedigree analysis was not possible for this patient.

Found substitutions were mentioned earlier in many papers reporting *vWF* gene conversion. According to HGMD, two pathogenic variants found in patient A cause the first type of vWD, which is consistent with the patients' A mild form of vWD. We sequenced a homologous part of the pseudogene for patients A and B and for patients' A mother. The patients' B pseudogene was identical to the reference (M60676.1, Mancuso et al., 1991), but pseudogene sequence in patient A had two heterozygous and two homozygous substitutions compared to the reference. Both heterozygous positions were located in the conversion area, and one of them was not usual for gene nor pseudogene. Patient' A mother had a pseudogene with all four of those substitutions in homozygous state. We sequenced the same pseudogene region for five control patients and all of them yielded sequences identical to the reference.

The mechanism of gene conversion in our cases is not clear because pseudogene has substitutions that are not characteristic for gene nor for pseudogene in patient A, and has untouched sequence (i.e. not carrying *vWF* gene nucleotides) in patient B.

Therefore, it was not an exchange of complementary parts of gene and pseudogene, and we cannot suggest any mechanism of conversion in this case.

Summary/Conclusion: Gene conversion took place in two cases out of 64, making its frequency in our sample to be 3,13%. The conversed gene allele in the heterozygous state caused mild form von Willebrand disease for patient A and carried only polymorphisms in patient B case. We assumed that gene conversion would be symmetrical, with gene and pseudogene swapping parts. However, it was not confirmed.

Keywords: von Willebrand's disease, von Willebrand factor (vWF), VWF