A large deletion identified in a Swedish family with type 1 VWD

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Dear Sirs,

von Willebrand disease (VWD) is a common inherited bleeding disorder and is caused by deficiency or abnormality of the von Willebrand factor (VWF) protein which is encoded by the VWF gene. The expression of type 1 VWD is influenced by modifying factors, the most well-known being blood group O (1). Three large studies that recently aimed to characterise the mutations causing type 1 VWD in Europe (2, 3) and in Canada (4) failed to identify causative mutations in 35–45% of the investigated cases. However, none of these studies (2–4) screened specifically for deletions. The ISTH SSC VWF database (http://www.vwf.group.shef.ac.uk/) lists 11 deletions as causative mutations for type 1 VWD mutations, except for one large deletion in May 2010, acting as recessive type 3 VWD mutations, except for one causing type 2 VWD. Recently, a deletion encompassing exons 4 and 5 has been identified in both type 1 and type 3 VWD (5).

In the present study we searched for deletions in the VWF gene using marker
data generated for other purposes. The marker method has been used to identify deletions as causative mutations in for example autism (6), congenital heart defects (7), and protein S deficiency (8). The logic behind the method is the following: If a deletion encompasses a genetic marker, an individual heterozygous for the deletion will appear to be homozygous for the other allele. Deletions covering a marker can thus be detected if a parent and a child appear to be homozygous for different alleles. This type of Mendelian inconsistency can be detected if a parent and a child appear to be homozygous for different alleles. However, the presence of such inconsistencies at several adjacent markers gives strong support for a deletion.

The genotype data from the two microsatellite markers in the study of Lanke et al. (9) and 171 single nucleotide polymorphism (SNP) markers were analysed for patterns compatible with a deletion in 40 three-generation families that have been investigated in another study of the VWF gene (C. Hallén, A.M. Johansson, S. Lethagen, and T. Säll, manuscript in preparation). The families were type 1 VWD families collected at University Hospital Malmö where samples from three generations were available. For patients where a deletion was identified, symptoms and test results on VWF:Rco and VWF:Ag were obtained from the patient records at University Hospital Malmö, Sweden. The ethical committee of Lund University and the Swedish Data Inspection Board approved the study and informed consent was obtained from all participating individuals.

The SNP and microsatellite marker segregation patterns identified 53 markers which showed homozygosity for different alleles in the grandchild (II:2), the mother (II:1) and the child (III:1) in family 554 (Fig. 1, see also Supplementary Table S1 available online at www.thrombosis-online.com). This is consistent with the presence of a large heterozygous deletion encompassing at least 107 kb, from the microsatellite in intron 15 to the SNP marker located after exon 52 (rs1990326), since it is extremely unlikely that this pattern would

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Figure 1: Inheritance patterns for three of the SNPs indicating the presence of a deletion in family 554. Like most of the 40 families, one child, two parents and one or two grandparents were genotyped in this family. The SNP markers are rs2238109 (intron 17), rs216867 (exon 42), and rs933408 (intron 51). A) The observed SNP genotypes. B) The interpreted true genotypes, del = deletion.
be due to genotyping errors or primer annealing site mutations in all 53 markers. Figure 1 illustrates the genotypes for three of the SNPs indicating a deletion. For example II:1 appears to be homozygous AA for rs2238109 and her son III:1 appears to be homozygous TT, which is a Mendelian inconsistency. This is however consistent with a deletion that is inherited from I:2 to II:1 and from II:1 to III:1, where III:1 inherited one T allele from his father who is heterozygous AT (Fig. 1B).

The mother (II:1) carrying the deletion was diagnosed with VWD and showed bleeding symptoms such as easy bruising, extensive nose bleeds and occasional gingival bleeds. Her VWF levels were low [VWF:RCo 0.47 kIU/l (normal range 0.76–2.29 kIU/l), VWF:Ag 0.49 kIU/l (normal range 0.60–2.73)]. Her son (III:1) that also carried the deletion was not diagnosed with VWD, although he had some bleeding symptoms such as easy bruising and gingival bleeds. He had moderately lowered VWF:Ag (0.47 and 0.57 kIU/l, measured on two occasions, normal range 0.60–2.73) and VWF:RCo values of 0.59 and 0.55 kIU/l, (normal range 0.52–1.58 kIU/l). The reason that this reference interval is different from the one above is that between these measurements the calibration shifted from 3rd IS to 4th IS. The grandfather (I:2) had the deletion and was not investigated using laboratory analyses, but had no self-reported bleeding symptoms. The grandmother (I:1) was deceased and not investigated, but had had bleeding symptoms.

Since the identified deletion is a rare mutation, identified in one out of 40 VWD families, it will rarely become homozygous but in homozygous state it is likely to cause type 3 VWD. Family 554 illustrates the complexity of type 1 VWD since only two out of three family members with the deletion have bleeding symptoms. The fact that the grandmother (I:1) also had had bleeding symptoms indicates that the mother (II:1) could have inherited some risk factor from her in addition to the deletion from the grandfather (I:2). None of the genotyped family members have blood group O, excluding this risk factor. Since the grandmother (I:1) is deceased it is difficult to resolve a possible additional risk factor in this family.

Additional Mendelian inconsistencies compatible with deletions or primer annealing site mutations were detected by 6 SNP markers, all located in introns (rs2191161, rs216296, rs216905, rs216891, g.138233 (111 bp into intron 40) and rs24177) in a total of 15 families. These Mendelian inconsistencies were either: i) not present on the haplotype co-segregating with disease, or ii) present in individuals where bona fide mutations had already been identified, or iii) present largely in unaffected individuals, or iv) confined only to introns. Taken together, this strongly indicates that none of these are disease-associated.

The recently reported deletion encompassing exons 4 and 5 of the VWF gene (5) is not detected in the present study. However, since only two of our SNPs are located in this region and as they both have low heterozygosity, our ability to detect this deletion is very limited.

This paper demonstrates the existence of a large deletion in the VWF gene, which clearly causes type 1 VWD, albeit with reduced penetrance. Overall, large deletions appear to be relatively uncommon in type 1 VWD.

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