An unexpected transmission of von Willebrand disease type 3: the first case of maternal uniparental disomy 12

Von Willebrand disease (VWD) is an inherited heterogeneous bleeding disorder caused by either a quantitative and/or qualitative defect of von Willebrand factor (VWF).[1,2] It is encoded by a gene located on chromosome 12p13.3 composed of 52 exons.[3] VWD type 3 involves a virtually complete quantitative VWF deficiency.[1] Its inheritance is autosomal recessive and is the result of the inheritance of two null alleles. The prevalence of type 3 is relatively low, with 0.5-1 cases per million in the general population.[4] Type 3 VWD, the most severe form of the disease, is characterized by a severe bleeding tendency, particularly in mucocutaneous sites.

A 3-year old girl was referred to our department for prolonged oral bleeding following a mouth wound. She had no medical history of abnormal bleeding and her birth had been normal. There was no familial history of hemorrhage. Physical examination revealed many bruises. Results of hemostatic tests of the propositus (Table 1) allowed diagnosis of VWD type 3. Paternal hemostatic tests were normal while the mother exhibited a mild deficiency of VWF, as is usually the case in carriers of VWD type 3.[5]

All patients gave their consent to carry out molecular genetic analysis. We screened patient DNA for mutations by direct sequencing of the exons of the *VWF* gene (NM_000552.3), using Big Dye V 3.1 and an ABI3130XL capillary sequencer (Applied Biosystems). Exon 20 was amplified using primers VWF-20F 5'AAGGTGCCCAACTTGTCATC3' and VWF-20R 5'CCCACCCCTCCTAGAAAGAA3'. The Human Genome Variation Society nomenclature (http://www.hgvs.org/mutnomen/) was used for the mutation. For the segregation study, PCR amplifications of 16 polymorphic markers on chromosome 12 were carried out with fluorescent primers. Six were on the *VWF* gene,[6-10] and the other 10 were located on the short and the long arms of chromosome 12 and were selected on the GeneLoc database (http://genecards.weizmann.ac.il/geneloc/index.shtml). The amplification products were resolved on the ABI3130XL sequencer and ana-

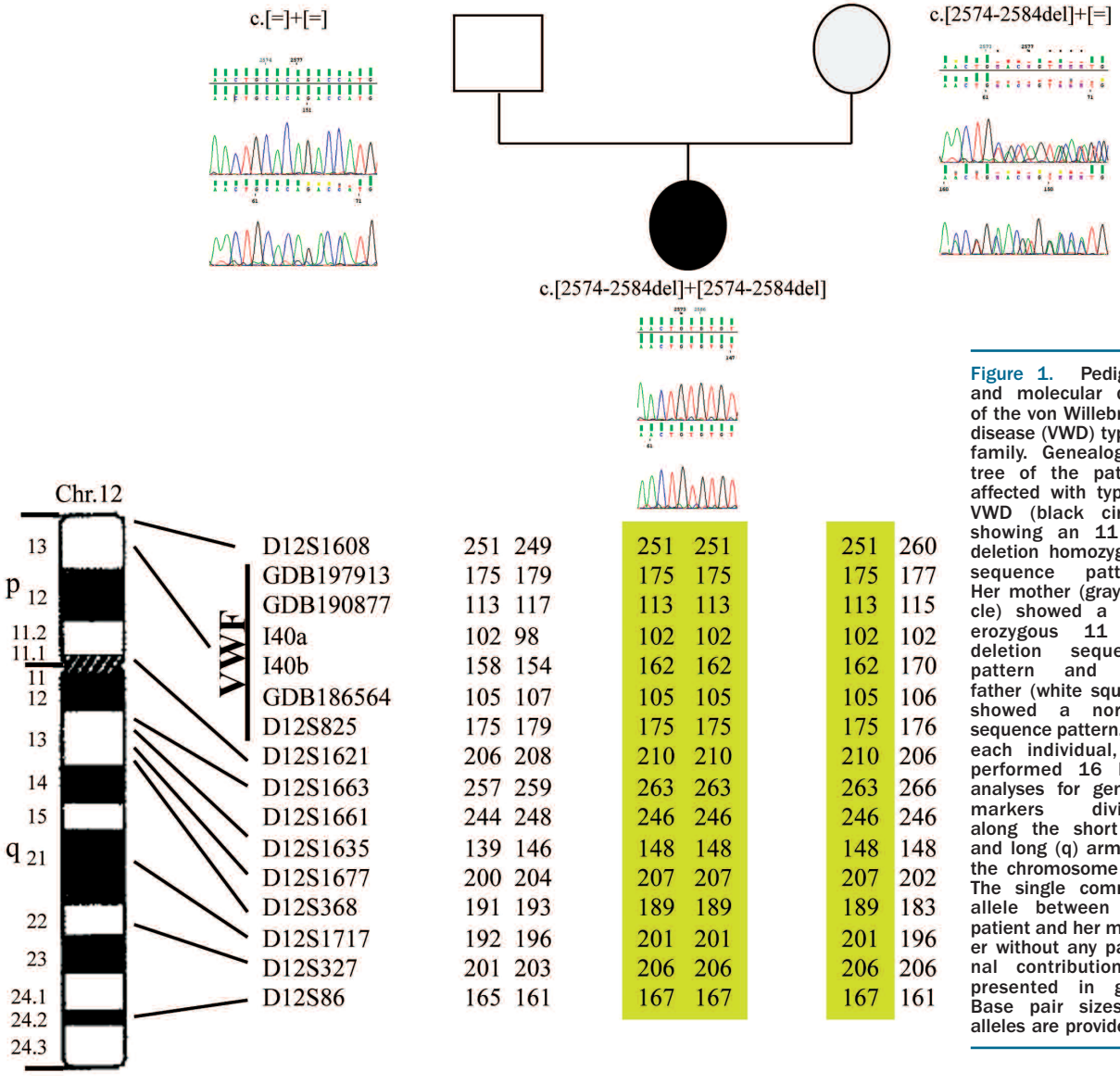

Figure 1. Pedigree and molecular data of the von Willebrand disease (VWD) type 3 family. Genealogical tree of the patient affected with type 3 VWD (black circle) showing an 11 bp deletion homozygous sequence pattern. Her mother (gray circle) showed a heterozygous 11 bp deletion sequence pattern and her father (white square) showed a normal sequence pattern. For each individual, we performed 16 PCR analyses for genetic markers divided along the short (p) and long (q) arms of the chromosome 12. The single common allele between the patient and her mother without any paternal contribution is presented in gray. Base pair sizes of alleles are provided.

Table 1. Results of hemostatic tests of the patient with VWD type 3 and her parents.

Hemostatic assays	Father	Propositus	Mother
Closure time (Col/Epi) (sec)	120	>300	192
Closure time (Col/ADP) (sec)	84	>300	144
APTT (sec - ratio)	35-1.06	73.6-2.23	37-1.1
Prothrombin index (%)	91	93	95
Fibrinogen concentration (g/L)	3.2	3.6	2.7
FVIII:C (IU/ml)	0.79	0.01	0.87
VWF:RCo (IU/ml)	0.99	<0.05	0.55
VWF:Ag (IU/ml)	0.90	<0.01	0.47
Platelet count $\times 10^9$/L	165	327	210
Blood group	0	0	0

lyzed with GeneScanv3.7 (Applied Biosystems).

We detected a homozygous 11pb deletion c.2574_2584del (Figure 1) on exon 20 of the *VWF* gene in the proband DNA. To our knowledge, this mutation has never been previously described or referenced to sequence databases. This c.2574_2584del results in a premature stop codon: p.Thr859ValfsX2. This frameshift mutation is clearly deleterious, leading to the truncation of the VWF protein or mRNA damage by nonsense mediated mRNA decay, which would explain the total plasma deficiency of VWF. The non-consanguineous parents of the patient were tested for the mutation by sequencing. We found a heterozygous 11pb deletion in the mother and, surprisingly, an absence of this mutation in the father. To test the hypothesis of uniparental disomy (UPD), 16 polymorphic genetic markers mapped to chromosome 12 were investigated. Every marker indicated a homozygosity of the entire chromosome 12 in the proband, consistent with maternal isodisomy.

To our knowledge, there has been no previous report of either paternal or maternal unidisomy involved in VWD. Interestingly, according to a recent review,[11] UPD of chromosome 12 has never been involved in a case of autosomal recessive disease.

This case highlights the need to perform extensive molecular analysis of the *VWF* gene in VWD type 3 patients in order to improve our knowledge of the molecular mechanisms and pathophysiology of the disease. Indeed, the discovery of a deletion indicates a high-risk factor for the occurrence of anti-VWF antibodies and may affect the therapeutic protocol for the patient. Moreover, identifying an association between UPD of chromosome 12 and VWD is important with regard to genetic counseling to inform parents about the negligible recurrence risk during a new pregnancy compared to the usual 25% risk related to an autosomal recessive transmission of type 3 VWD.

Pierre Boisseau,[1,5] Mathilde Giraud,[1,5] Catherine Ternisien,[2,5] Agnès Veyradier,[4,5] Edith Fressinaud,[4,5] Armelle Lefrancois,[1,5] Stéphane Bezieau,[1,3,5] Marc Fouassier[2,5]

[1]CHU Nantes, Service de Génétique Médicale, Nantes, 44093; [2]CHU Nantes, Centre de Traitement des Hémophiles, Nantes, 44093; [3]Université de Nantes, Faculté de Médecine, Laboratoire Biométadys EA4273, 1 rue Gaston Veil, Nante; [4]Service d'hématologie biologique, Hôpital Antoine Béclère, Clamart; [5]Centre National de Référence de la Maladie de Willebrand, France.

Correspondence: M. Fouassier, Centre de Traitement des Hémophiles, Centre Hospitalier Universitaire de Nantes, 9, Quai Moncousu, 44093 Nantes Cedex 1, France. Phone international 033240084049; fax: international 033240084026; E-mail: marc.fouassier@chu-nantes.fr;

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Minimal residual disease-directed preemptive treatment with azacitidine in patients with NPM1-mutant acute myeloid leukemia and molecular relapse

Therapeutic options are often limited in patients with acute myeloid leukemia (AML) who relapse after inten-