

**Description of family with co-occurrence of von Willebrand disease and  
mood disorders.**

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## ABSTRACT

This report is an addendum to a brief report in *Bipolar Disorders* (von Willebrand disease and psychotic disorders: co-segregation and genetic associations; Lee et al., see our website for a copy). We report here on an extended family that led to the investigation of *vWF*, the gene conferring susceptibility to von Willebrand disease (vWD). The nuclear family that forms the core of the pedigree includes four members with vWD and mood disorders. Co-segregation was not evident in the extended pedigree. The structure of the pedigree has been changed to protect confidentiality. Reports of families with similar co-occurrence are welcome.

## INTRODUCTION

The etiology of mood disorders, and in particular mood disorders with psychotic features, is unknown. Insight may be gained by investigation of suitable families cosegregating mood disorders and diseases with known etiology. Such investigations may yield clues about genetic as well as non-genetic etiological factors. By way of illustration, we describe a family which includes several members diagnosed with mood disorders and type 1 von Willebrand disease.

von Willebrand disease (vWD) is the most common inherited bleeding disorder in humans. Its prevalence has been estimated at 0.82-1.6%, but this may be an underestimate due to difficulties in establishing laboratory based diagnoses [Rodeghiero et al. 1987]. It is characterized by distinct quantitative and / or qualitative abnormalities of von Willebrand factor (vWF), a large multimeric glycoprotein with two key roles in hemostasis. vWF serves as a carrier protein of factor VIII, an essential cofactor in coagulation. It also promotes platelet adhesion to the damaged vessel wall, which is the initial step of primary haemostasis [Mazurier et al., 1998]. Therefore, patients who lack vWF can have profound defects both in blood clotting and in the formation of platelet plugs at sites of vascular injuries [Sadler, 1998]. Patients with vWD may be asymptomatic or may present with epistaxis, menorrhagia, excessive post-operative bleeding, or recurrent minor bleeds.

vWD has been classified into three main groups based on qualitative or quantitative deficiencies of vWF: types 1, 2 and 3 [Sadler, 1994]. Patients with Type 1 vWD have a quantitative deficiency of vWF with normal multimeric structure and

function. It accounts for 70-75% of all vWD patients and has an autosomal dominant pattern of inheritance [Mazurier et al., 1998]. The vWF gene has been localized to chromosome 12p12-pter and its genomic structure has been determined [Ginsburg et al., 1985]. It includes 52 exons and spans approximately 180 kb [Mancuso et al., 1989]. Over 33 mutations in the vWF gene have been reported to date [Sadler and Ginsburg, 1993]. Asymptomatic carriers of *vWF* mutations are also known [Sadler and Davie, 1994; Rodeghiero et al., 1987]. To our knowledge, familial co-segregation of vWD with mood disorders, as reported here, has not been described to date.

## METHODS

Clinical The multiply affected family was ascertained in the course of our ongoing genetic studies of schizophrenia and bipolar disorder. We obtained written informed consent according to the guidelines of the University of Pittsburgh Institutional Review Board (IRB). Consenting individuals were interviewed using the Diagnostic Interview for Genetic Studies (DIGS) [Nurnberger et al., 1994]. The DIGS is a structured interview schedule which includes questions related to lifetime and current occurrence of common psychiatric conditions. Additional clinical information was obtained from available clinical records and from other relatives as required. This information was synthesized and a consensus diagnosis assigned by two psychiatrists using DSM-IV criteria. Both psychiatrists were blind to the identity of the individuals as well as their hematologic status. The Family Interview for Genetic studies, a semi-structured scale developed by the National Institute of Mental Health Collaborative Genetics Initiative, was used to obtain family history of psychiatric and non-psychiatric illnesses.

## **Laboratory**

Hematological analyses Venous blood was collected in 3.2% sodium citrate tubes from consenting members of the multiplex family and the following diagnostic laboratory tests for vWD conducted. Enzyme-linked immunosorbent assays (ELISA) were used for analysis of vW antigen [Silveira et al., 1986] and Ristocetin cofactor activity was determined using a modified commercial platelet agglutination method (Dade Behring, Deerfield, IL). Multimeric analysis was performed on abnormal vW subjects by sodium dodecyl sulfate (SDS) electrophoresis of plasma according to the method of Ruggeri et al. [Ruggeri et al., 1980]. The multimeric protein bands are electrophoretically separated in agarose by molecular weight. Hematological investigations were not conducted among the simplex families (case-parent duos and trios) or the neonatal controls.

Molecular genetic analyses Genomic DNA was isolated from venous blood samples using the phenol-chloroform method. To enable haplotype based analyses, we selected three *vWF* single nucleotide polymorphisms (SNPs) from over 33 polymorphisms identified to date [Sadler and Ginsberg, 1993]: 1548T>C (exon 14, detectable with *Acc* I) [Kunkel et al., 1991], 2365A>G (exon 18, *Rsa* I) [Kunkel et al., 1990] and an *Msp* I polymorphism in intron 19 (C/G) [Mercier et al., 1990]. All three SNPs have minor allele frequencies exceeding 0.2 among Caucasians, and none occur in the *vWF* pseudogene sequence, localized to 22q11. Since these SNPs have been used in prior linkage analyses involving vWD pedigrees, we used them to define informative sets of haplotypes. Allelic variation was examined using PCR based amplification followed by

restriction enzyme digestion and electrophoresis on 2% agarose gels stained with ethidium bromide. PCR were performed using a PTC 100 thermal cycler (M J Research). The PCR reactions (10  $\mu$ l) included 60ng genomic DNA , 200uM dNTP, 1.5 mM MgCl<sub>2</sub>, 6 picomoles of each primer , 0.25 U Taq polymerase, and 1X PCR buffer (2.5 mM MgCl<sub>2</sub> for T1548C). The PCR conditions involved initial denaturation at 96 C for 5 min, 31 cycles of 96 C for 30 sec, 54 C for 30 sec, 72 C for 1 min, and a final extension at 72 C for 10 min (annealing temperature 54 C for *Msp* I polymorphism). In our nomenclature, allele '1' denotes T1548 at 1548T>C, A2365 at 2365A>G and the digested product at the intronic polymorphism respectively.

#### Data analysis

Statistical analysis The family was checked for Mendelian inconsistencies using PEDCHECK software [O'Connell and Weeks, 1998].

### **Results**

#### Multiplex family

Using the DIGS, psychiatric evaluations and laboratory investigations for vWD were completed for the proband, all his first degree relatives and other available relatives. One maternal aunt and the maternal uncle did not report any psychopathology, and did not have a history of bleeding, but hematologic evaluation could not be conducted for these individuals (please see Figure 1 for the pedigree structure). A detailed description of psychopathology among affected members follows and the hemotological investigations are listed in Table 1.

The proband was diagnosed with depression and prescribed antidepressants when he was 24 years old. Over the next eight years, he had ten major depressive episodes and six manic episodes (DSM IV criteria; American Psychiatric Association, 1994). In addition, he had psychotic features including paranoid delusions, thought insertion, thought withdrawal, and auditory hallucinations. These phenomena occurred both during depressive and manic episodes. He has been treated on an outpatient basis and has never required psychiatric hospitalization. He received a consensus diagnosis of schizoaffective disorder, bipolar type.

The proband's second sibling (#2) was prescribed amphetamine for hyperactivity when she was 9 years old. She also abused marijuana for 11 years. A consensus diagnosis of attention deficit hyperactivity disorder was made, although she was diagnosed with bipolar disorder at the age of 14 by her physician.

The proband's sister (#3) reports more than ten major depressive and manic episodes between the ages of 10 and 24. She first received anti-depressants when she was 15 years old. She has also experienced grandiose, jealous, referential and persecutory delusions, as well as auditory, somatic and visual hallucinations. She also exhibited alogia, inappropriate emotions, derealization and depersonalization. She received a consensus diagnosis of schizoaffective disorder, bipolar type. She has also been diagnosed with nephrocalcinosis and medullary sponge kidney.

The proband's mother first experienced depressive symptoms in her teens. At the age of 18, she was hospitalized following a suicide attempt and depression. She has also experienced manic episodes characterized by elevated mood, psychomotor agitation, increase in goal-directed activity and talkativeness. She received a consensus diagnosis of

bipolar I disorder, most recent episode unspecified. She also has fibromyalgia, asthma and allergy. She reported aspirin and perinatal hemorrhage during all her pregnancies. She was prescribed amphetamines during her first pregnancy for weight control and for six months post-natally.

The proband's father reports onset of psychosis at the age of nine, with vivid auditory hallucinations. He has suffered both auditory and visual hallucinations intermittently since then. He also reports approximately 15 depressive and 4 manic episodes. He does not currently abuse alcohol, though there is a decade long past history. He received a consensus diagnosis of schizoaffective disorder, bipolar type.

The proband's maternal aunt (#5) reported depressive episodes and had an extensive history of substance abuse including cocaine, stimulants, sedatives, opioids, hallucinogens, solvents. She was diagnosed with major depressive disorder, severe without psychotic features, and with polysubstance dependence.

The proband's male second cousin (#10) reports 15 episodes of depression, with onset at 12 years of age. He was diagnosed with depressive disorder NOS. He also has a history of grand mal seizures, which began when he was 3 - 4 years old. He received anti-convulsant drugs for the next seven years.

The proband's female second cousin (#11) reported symptoms of psychosis such as persecutory delusions and delusions of reference, as well as visual, auditory and olfactory hallucinations from 13 years of age. In addition, she reported two severe episodes of depression which began when she was 12 and lasted for 3 years. She has abused alcohol, stimulants, cocaine, hallucinogens, and solvents. She received a consensus diagnosis of

schizoaffective disorder, depressed type. Laboratory analysis excluded a diagnosis of vWD.

#### Analysis of vWF polymorphisms in the multiplex pedigree

The individuals listed in Table 1 were also genotyped for three SNPs at *vWF* as described. The haplotypes were uninformative as all but one individual was monomorphic for all three SNPs. Notably, the haplotype transmitted to the proband and his siblings was identical and included alleles of SNPs that showed transmission distortion in the nuclear families (i.e., allele '2' at 1548T>C, allele '1' at 2365A>G and allele '2' at the *Msp* I polymorphism).

## DISCUSSION

The multiplex family described here includes four members with vWD and others with bleeding diatheses of unknown etiology. Since the proband, his siblings and his mother also received concurrent diagnoses of psychiatric disorders (predominantly mood disorders), we investigated the co-segregation of vWD and mood disorders in the extended pedigree. The psychiatric illnesses present among the relatives included a number of disorders, with mood abnormalities and psychotic features being the most common. It appears unlikely that the *vWF* gene has a direct etiological role for mood disorders in this family, since three other relatives had mood disorders but were not diagnosed with vWD. Since the proband's father was diagnosed with schizoaffective disorder and several third degree relatives did not complete psychiatric evaluations,

precise estimates for the likelihood of co-segregation of vWD and any of these disorders cannot be obtained.

Haplotype based analyses in this family were also uninformative due to unusual levels of homozygosity. The homozygosity among all three SNPs may be explained by the significant LD observed in the population based sample.

There may be non-genetic explanations for the unusual familial clustering. For example, the proband's mother reported aspirin usage and perinatal bleeding during all her pregnancies, increasing the likelihood of minor cerebral hemorrhage in the fetuses [Huttunen et al., 1994]. Such hemorrhages, as well as bleeding episodes due to vWD among the proband and his siblings during the intra-uterine or perinatal period could have increased the risk of psychiatric illness [Lyon and Barr, 1991]. Interestingly, others have reported co-segregation of mood disorders and Christmas disease, a bleeding disorder due to factor IX deficiency [Gill et al., 1992] [Craddock and Owen, 1992].

In summary, a family with unusual co-occurrence of vWD and mood disorders is described. Though the familial clustering of psychiatric and bleeding disorders is uncommon, chance co-occurrence can not be excluded in this pedigree. Novel non-genetic explanations for the co-occurrence are also plausible. Thus, the question of a genetic association between *vWF* and BD I is unresolved. Reports of other families with similar co-occurrence are welcome.

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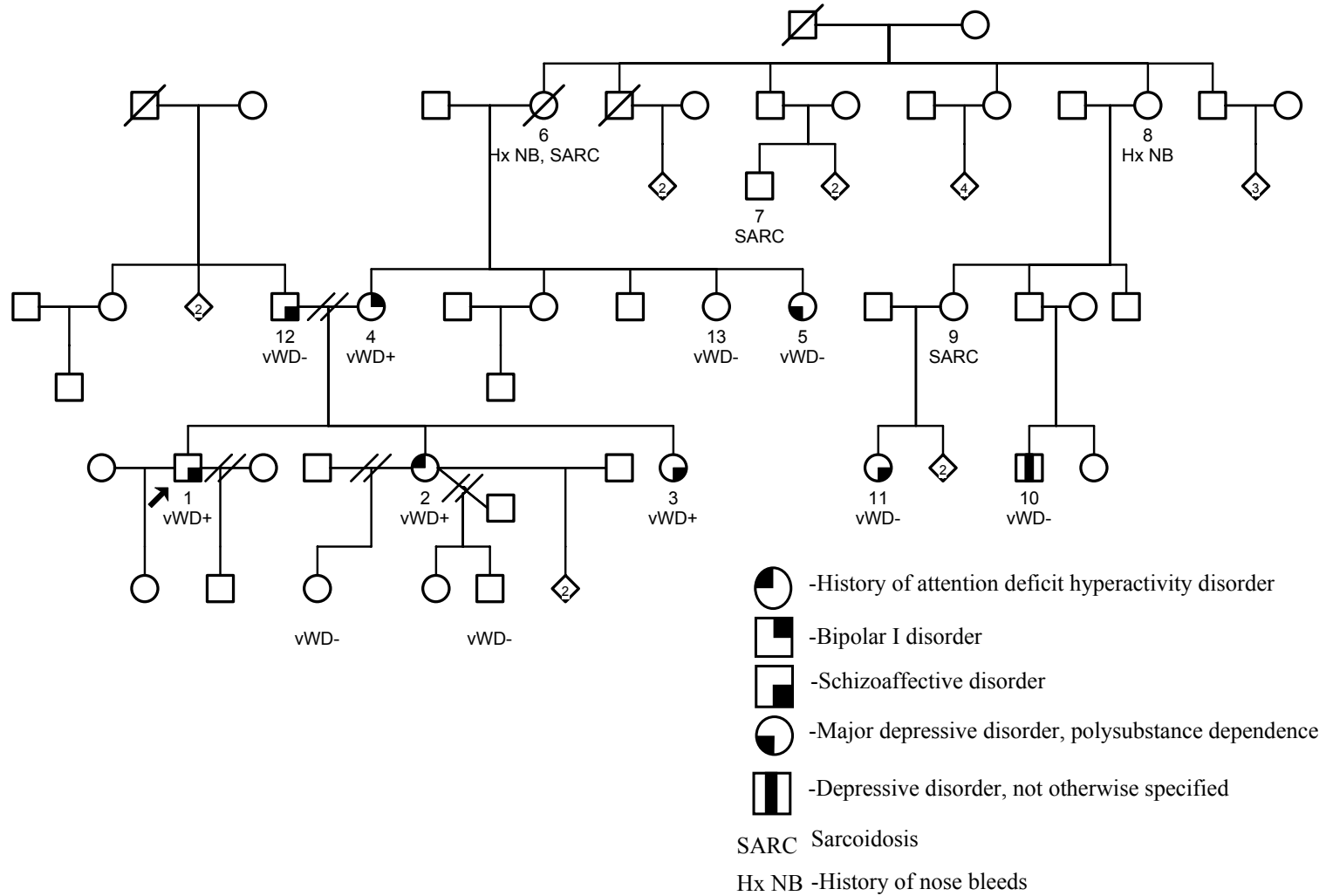
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**Figure 1. Pedigree Structure and diagnoses**



**(Legend for Figure 1).**

To protect confidentiality the structure of the pedigree has been changed. The numbers refer to identification numbers. vWD+ = von Willebrand's disease (vWD) present; vWD-: vWD absent, following laboratory analyses. Individuals without these symbols were not evaluated hematologically. Except where indicated none of these individuals had a history of nose bleeds.

**Table 1. Hematological investigations and psychiatric diagnoses**

Subject (ID no)	APTT (s)	APTT mix filtered (s)	Factor VIII:C (U/ml)	vW Antigen (U/ml)	Ristocetin co-factor (IU/ml)	vW Antigen mutlimers	Diagnosis of vWD?	Diagnosis
	(26.0-36.0)	(32.0-42.0)	(0.50-1.50)	(0.78-1.53)	(0.50-1.50)			
Proband (1)	35.4	39.4	0.92	0.65	0.65	normal	yes	SAD
Proband's sister (2)	33.2	36.7	1.00	0.62	0.51	normal	yes	ADD
Proband's sister (3)	36.2	41.7	0.64	0.44	0.27	normal	yes	SAD
Proband's mother (4)	28.7	38.7	1.39	0.70	0.49	normal	yes	BD I
Proband's father (12)	34.2	39.2	0.56	1.10	0.78	normal	no	SAD
Proband's maternal aunt (5)	33.7	38.9	1.36	1.22	1.52	NA	no	MDD
Proband's maternal aunt (13)	47.9	38.7	0.84	0.87	0.81	NA	no	-
Proband's male second cousin (10)	33.2	37.6	0.96	1.61	1.86	NA	no	DNOS
Proband's female second cousin (11)	37.7	37.6	0.64	0.79	0.54	NA	no	SAD

The numbers in the second row refer to reference ranges for the relevant investigations. The numbers in parentheses refer to the identification numbers used for the pedigree in Figure 1.

SAD: schizoaffective disorder; ADD: attention deficit hyperactivity disorder; BD I: bipolar I disorder; MDD: major depressive disorder; DNOS: depressive disorder, not otherwise specified; NA: not analyzed.



