Simultaneous inheritance and expression of classical haemophilia A and type IIA von Willebrand's disease

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SUMMARY A family is described in which the mother is a haemophilia carrier, the father has asymptomatic type IIA von Willebrand's disease, and their second son has simultaneously inherited both severe haemophilia and type IIA von Willebrand's disease. This is the first report of both diseases occurring simultaneously. The inheritance patterns and laboratory data on the family are presented and discussed.

Von Willebrand's disease is a heterogeneous group of autosomally transmitted bleeding disorders, which are characterised clinically by bruising and mucous membrane bleeding and by the laboratory findings of quantitative or qualitative abnormalities of factor VIII activities (FVIII procoagulant, VIII:C; related antigen, VIII:R:AG; von Willebrand factor, VIII:R:WF).^{1,2} Recently, a number of variant (type II) forms of von Willebrand's disease have been characterised by multimeric analysis of the von Willebrand protein (VIII:R:AG/VIII:R:WF) and by the quantitative aggregation of patients' platelets by ristocetin.^{3,4}

In contrast, classical haemophilia A is inherited as an X linked recessive disorder and is characterised in its severest form by recurrent spontaneous haemarthroses and muscle bleeds. VIII:C values are low, but levels of VIII:R:AG and ristocetin induced platelet aggregation are usually normal.⁵ The inherited coagulation disorders are uncommon. The estimated incidence of classical haemophilia is 5-10 per 100 000 population.⁶ Estimation of the incidence of von Willebrand's disease is difficult because of the existence of asymptomatic heterozygotes and its variable clinical expression.

We have recently identified a patient who has simultaneously inherited both classical haemophilia A and type IIA von Willebrand's disease. This is the first report of documented von Willebrand's disease and classical haemophilia in the same individual. The clinical, including inheritance pattern, and laboratory features are discussed.

Material and methods

SUBJECTS

The proband was referred because he had bled excessively from a torn frenulum and developed a large scalp haematoma after a small knock. There was a known maternal family history of haemophilia (Fig. 1). The family is of Afrocaribbean origin and

Fig. 1 Family pedigree. Proband indicated by arrow.

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Results of coagulation studies

Initial	Relationship	Ivy bleeding time (min)	PTT (s)	VIII:C (U/ml)	VIII:AG (U/ml)	VIII:WF (U/ml)	Migration ratio (by crossed immunoelectrophoresis)
CC	Propositus	4.5	74	0.00	0.78	0.05	1.50
LC	Father	3	31	0.72	1.76	0.05	1.30
DC	Mother	3	35	0.42	1.20	0.90	1.00
CC	Brother	3	34	0.90	1.00	0.90	1.00
Normal range		up to 7	32	0.5-1.50	0.5-1.50	0.5-1.50	0.99±0.11

PTT = partial thromboplastin time

the propositus' mother was related through her maternal grandmother to another local family who have a son with severe haemophilia (VIII:C 0 U/ml; VIII:AG 1.80 U/ml; VIII:WF 0.66 U/ml). The propositus' mother had a brother who died, as a child, of a cerebral haemorrhage in Jamaica.

The propositus' father and brother had no clinical symptoms to suggest a bleeding disorder. The propositus' father, however, had not had any operations or extractions of his permanent dentition. The father, brother, mother, and propositus were studied, but the father's relatives were unfortunately not available for testing.

PLATELETS AND PLASMA

Venous blood was obtained from family members and normal men as controls. Nine parts of blood were added to one part of 0.11 M sodium chloride. Platelet rich plasma was prepared by centrifuging 150 g for 10 min at room temperature and platelet poor plasma was prepared by centrifuging at 2700 g for 15 min at 4°C. The standard for the factor VIII procoagulant activity (VIII:C) was a 100% reference plasma (Immuno Diagnostika) calibrated against the 10th British Factor VIII standard (VIII:C 0.65 IU/ml); the standard for the factor VIII related antigen (VIII:AG) was the 10th British Standard (VIII:AG 0.80 IU/ml) and a pool of plasma from 30 normal men (VIII:AG 1.0

IU/ml). The latter was also used as the standard for the von Willebrand factor (VIII:WF) assay. Platelets were washed with EDTA tris buffer and concentrated by centrifuging on to 34% wt/vol bovine albumin.⁷ Platelets were washed four times before fixing with paraformaldehyde and used in the VIII:WF assay (see below).

Ristocetin was supplied by Lundbeck A/S, Denmark; and agarose was supplied by BDH Poole, Dorset. Rabbit antihuman VIII serum was supplied by Behringwerke and used in the VIII:AG crossed immunoelectrophoresis. Another antiserum (Dako rabbit antihuman FVIII immunoglobulin) was used unconjugated and conjugated in the VIII:AG ELISA assay. The same antibody labelled with ¹²⁵I was used for identifying the VIII:AG multimers.

Bleeding time was measured by the modified Ivy technique. VIII:C was measured by a two stage assay using a Diagen kit (Diagnostic Reagents Ltd). VIII:AG was measured by an ELISA technique;⁸ and VIII:AG electrophoretic mobility was assessed by crossed immunoelectrophoresis.⁹ VIII:AG multimeric analysis was done as previously described¹⁰ using a modification of the method of Ruggeri and Zimmerman.¹¹ VIII:WF was measured using fixed platelets as described by Brinkhous and Read.¹²

Platelet aggregation studies were performed only on the father's platelet rich plasma, adjusted to a

Fig. 2 Crossed immunoelectrophoresis of VIII:AG on propositus (CC), father (LC), mother (DC), brother (AC), and normal subject (NP). Directions of electrophoresis as shown. Figure below each arc indicates degree of migration (in mm) from centre of well to peak of precipitin arc.

platelet count of $300 \times 10^9/l$, using a dual channel aggregometer (Malin Electronics Ltd, Ayr, Scotland). Studies were performed with adenosine diphosphate (BDH), collagen (Hormon-Chemie, Munich), ristocetin (final concentrations 1.25 and 1.5 mg/ml), bovine fibrinogen (final concentration 1.7 mg/ml) and thrombin (final concentration 1.7 units/ml). Aggregation patterns were compared with those of platelet rich plasma from a normal control tested under identical conditions.

Results

The pedigree and results of the coagulation studies are shown in Fig. 1 and the Table, respectively.

The propositus (CC) had VIII:C and VIIIIR:AG values typical of severe haemophilia, but was unusual in that he lacked VIIIIR:WF. His mother (DC) had a normal VIIIIR:AG value but reduced VIII:C, with a VIII:C and VIIIIR:AG ratio of 0.32, similar to that seen in haemophilia carriers.¹³ She, unlike her son, had a normal VIIIIR:WF value, whereas the father (LC) had almost absent VIIIIR:WF despite normal VIII:C and VIIIIR:AG values. Platelet aggregation studies on platelet rich plasma from the father (LC) were normal with collagen, adenosine diphosphate, bovine fibrinogen, and thrombin, but absent with ristocetin.

Although VIIIIR:AG values were normal in all family members, crossed immunoelectrophoresis patterns were abnormal for the propositus and his father. In plasma from these, an abnormal precipitin arc characterised by a decrease in the electrophoret-

ically slower high molecular weight VIIIIR:AG forms and a more anodal peak were seen (Fig. 2). Migration ratios (ratio of anodal migration of test to control) were also raised.

Multimeric analysis confirmed the absence of the larger and intermediate molecular weight multimers in the propositus and his father (Fig. 3), but the mother and brother had normal multimeric analysis. The absence of high and intermediate multimers is typical of type IIA von Willebrand's disease.^{3,11} The father therefore appears to have asymptomatic type IIA von Willebrand's disease, while his son has this and haemophilia. In normal plasma, the lower bands consist of a triplet configuration (Fig. 3). Those of the propositus and his father contrast with those of the normal subject (NP), the mother (DC), and the brother (AC) in that the central band and the a and b sub-bands of the triplet were more densely staining. VIIIIR:AG multimeric analysis for the mother (DC) and the brother (AC) was normal.

Discussion

Type II von Willebrand's disease is characterised by a qualitative defect of the von Willebrand protein (VIIIIR:AG/VIIIIR:WF) detectable by crossed immunoelectrophoresis and sodium dodecyl sulphate agarose gel electrophoresis. Recently, patients with type II von Willebrand's disease have been subdivided.^{3,4} The IIA variants show an absence of large and intermediate VIIIIR:AG multimers in both plasma and platelets;^{3,11} VIIIIR:WF and ristocetin induced platelet aggregation are absent or low. The

Fig. 3 VIIIIR:AG multimeric analysis patterns for the propositus (CC), father (LC), mother (DC), and brother (AC) and normal subject (NP). Direction of electrophoresis as shown; upper arrow indicates gel interface and position of IgM molecular weight marker as shown.

type IIB variants lack the large multimers in their plasma but not in the platelets, and ristocetin induced platelet aggregation occurs with normal and subnormal ristocetin concentrations.³ In both IIA and IIB variants, as well as in normal plasma, each multimer has a triplet configuration represented by a central band flanked by two subsidiary bands (designated b below and a above the central band). Although the mobility of the triplets in IIA, IIB, and normal plasma is the same,^{11,13} differences in triplet configuration are seen between different IIA patients and normal subjects.^{11,14} A further variant, type IIC, has recently been described.⁴ This condition is inherited in an autosomal recessive way, and as well as lacking the larger multimers, the multimer mobility is different and there is a doublet rather than a triplet configuration.

The proband and his father fulfil some of the criteria for type IIA von Willebrand's disease. The proband, but not his brother, has inherited this disorder, showing an autosomal dominant pattern. The proband appears also to have inherited severe haemophilia from his mother. The normal bleeding time of the proband and his father is most unusual in the light of findings indicative of type IIA von Willebrand's disease. This may partly explain the lack of previous abnormal bleeding in the father, although, as yet, he has not had any operative challenge. Measurements of bleeding time may vary considerably in the same individual,¹⁵ but on repeated testing, the proband and his father have always had normal bleeding times. Disparity between the bleeding time and *in vitro* VIII:WF assays in von Willebrand's disease has been reported,¹⁶ and measurement of VIII:WF based on *in vitro* ristocetin cofactor assays may not always reflect *in vivo* biological function.¹⁷

Absence of the largest VIII:AG/VIII:WF multimers, as in type IIA patients, is associated with a bleeding diathesis.^{18,19} Although the high and intermediate multimers are absent in the father's plasma, he has had no abnormal bleeding symptoms. Different portions of the VIII:AG/VIII:WF protein may be involved in several platelet related phenomena²⁰ and the exact relation between these functions remains unclear.¹⁷ The role of VIII:AG/VIII:WF function in platelet adhesion to the subendothelium is complex.²¹

Men with haemophilia inherit VIII:C deficiency from their carrier mothers. Haemophiliacs have normal VIII:AG and VIII:WF synthesis and function.¹⁷ Carrier females have normal VIII:AG values but VIII:C may be reduced because of random inactivation of normal and abnormal X chromosomes as postulated by Lyon. Identification of a proportion of haemophilia carriers can be made

on the basis of VIII:C:VIII:AG ratios.¹² The proband in this report has inherited haemophilia from his carrier mother and, although he shows a normal VIII:AG value, this is qualitatively abnormal (low VIII:AG, abnormal multimer pattern). As the father shows similar VIII:AG/VIII:WF abnormalities, the father's gene is being expressed in a dominant way, while the mother's normal gene for VIII:AG is not expressed.

Haemophilia A and von Willebrand's disease in the same kindred has been reported previously. Quick and Adlam²² reported a family with haemophilia A in which the presence of von Willebrand's disease was suggested by clinical bleeding pattern, inheritance pattern, prolonged bleeding time, and a positive tourniquet test. No individual was known to have inherited both disorders. Geiger and Rath²³ reported a kindred with factor VIII deficiency in which von Willebrand's disease was inferred because of autosomal inheritance and prolonged bleeding time. Two children were thought to have both disorders because of clinical symptoms and prolonged bleeding times, although the latter was occasionally normal. Both of these studies, however, were performed before the availability of assays for VIII:AG, VIII:WF, and quantitative platelet agglutination with ristocetin and therefore the claim cannot be fully substantiated. The possibility of aspirin ingestion causing the prolonged bleeding time cannot be excluded, and prolonged bleeding times have been recorded in haemophiliacs.²⁴ Holmberg and Nilsson reported several cases of well documented haemophilia A and von Willebrand's disease in a Swedish family, but no individual had both diseases.²⁵ A more extensive clinical and laboratory investigation of a family was reported by Gastaldi *et al.*²⁶ Although individuals with either haemophilia or von Willebrand's disease were identified, none had both diseases simultaneously.

The proband in the family described in this paper shows clear simultaneous inheritance, substantiated by laboratory data, of both haemophilia and type IIA von Willebrand's disease. As this boy is still young, it is not yet clear if he will have a more severe bleeding problem than if he had only inherited one of these diseases.

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