hereditary elliptocytosis and hereditary pyropoikilocytosis. *Blood Cells Mol Dis* 1996;22:254–8.
8 Delaunay J. The hereditary stomatocytoses: genetic disorders of the red cell membrane permeability to monovalent cations. *Semin Hematol* 2004;41:165–72.
9 Carella M, Stewart G, Ajetunmobi JF *et al.* Genomewide search for dehydrated hereditary stomatocytosis (hereditary xerocytosis): mapping of locus to chromosome 16 (16q23-qter). *Am J Hum Genet* 1998;63:810–6.
10 Fricke B, Argent AC, Chetty MC *et al.* The 'stomatin' gene and protein in overhydrated hereditary stomatocytosis. *Blood* 2003;102:2268–77.
11 Beutler E. G6PD deficiency. Review. *Blood* 1994;84:3613–36.
12 Dalal BI, Kollmannsberger C. Drug-induced haemolysis and methaemoglobinaemia in glucose-6 phosphate dehydrogenase deficiency. *Br J Haematol* 2005;129:291.
13 Beutler E, Baronciani L. Mutations in pyruvate kinase. Review. *Hum Mutat* 1996;7:1–6.
14 van Wijk R, Rijksen G, van Solinge WW. Molecular characterisation of pyruvate kinase deficiency – concerns about the description of mutant PKLR alleles. *Br J Haematol* 2007;136:167–9.
15 Kutlar A. Sickle cell disease: a multigenic perspective of a single gene disorder. Review. *Haemoglobin* 2007;31:209–24.
16 Vichinsky E. New therapies in sickle cell disease. Review. *Lancet* 2002;360:629–31.
17 Williamson D. The unstable haemoglobins. Review. *Blood Rev* 1993;7:146–63.

Von Willebrand disease

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Von Willebrand disease (vWD) is the most common of the inherited bleeding disorders, with a prevalence of symptomatic vWD around 125 per million.[1] It is caused by mutations affecting the von Willebrand factor (vWF) gene which result in either quantitative or qualitative abnormalities of vWF.

Patients with vWD have a mucocutaneous pattern of bleeding, with easy bruising associated with trauma and may report spontaneous bruising. Epistaxes and menorrhagia are common features. Prolonged bleeding following haemostatic challenges, including minor cuts, dental extractions and surgical procedures, is also typical.

vWF is synthesised predominantly in blood vessel endothelial cells but is also produced by bone marrow megakaryocytes and incorporated into platelet alpha granules.[2] The vWF gene is located on chromosome 12. The primary gene product is a protein with a molecular weight of approximately 250 kDa. Within cells of synthesis the vWF protein undergoes polymerisation, forming a range of multimers up to 80 protein units in size. vWF multimers are secreted from blood vessel endothelial cells directly into the circulation or are retained in the cells in specialised storage granules called Weibel-Palade bodies from which they are released as a response to vascular injury.

The main function of vWF is to facilitate platelet binding to blood vessel subendothelium at sites of vascular damage in high shear flow environments such as small arteries and the microvasculature (Fig 1).[3] Once platelets are anchored in place, vWF enables aggregation of platelets recruited to the site of injury culminating in the formation of a haemostatically effective platelet plug. The high molecular weight multimer forms of vWF are required for these platelet interactions.

vWF also functions as the carrier protein for clotting factor VIII (FVIII), protecting it from proteolytic degradation in the circulation and transporting it to sites of vascular injury to enable it to carry out its crucial role in coagulation. Qualitative and quantitative defects in vWF result in impairment of the haemo-

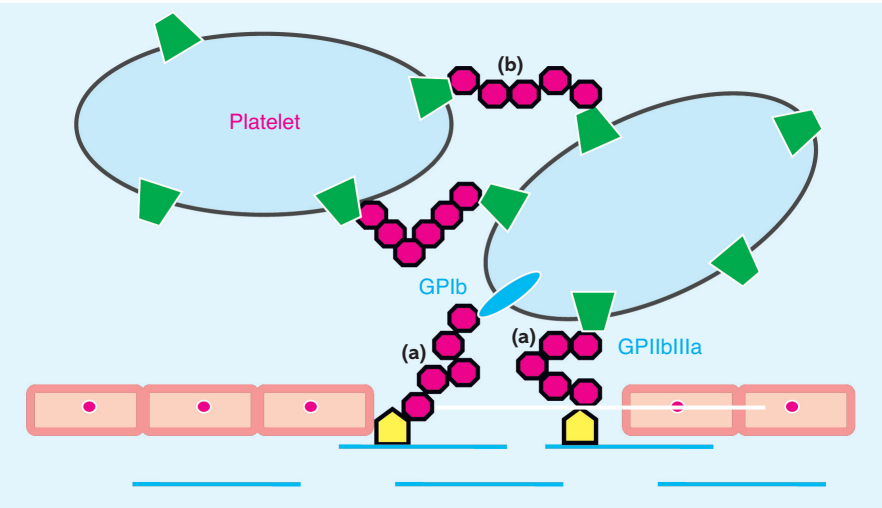

Fig 1. Role of von Willebrand factor (vWF) in haemostasis: (a) binding of vWF multimers to subendothelial collagen with capture of platelets, initially by binding to the glycoprotein (GP) Ib receptor and then to the GPIIbIIIa receptor; (b) aggregation of platelets by interbridging of vWF multimers between GPIIbIIIa receptors. Each vWF molecule can bind a factor VIII molecule (not shown) which is released at the site of haemostasis activation by the action of thrombin.

Table 1. Laboratory investigations of von Willebrand disease (vWD).

Investigation	Reason
Full blood count	To check for thrombocytopenia which may be present in type 2B vWD (*see below*)
Activated PTT	May be prolonged, indicating reduced FVIII levels
vWF antigen level*	To assess total amount of vWF protein
vWF activity level*	To assess activity of vWF by the RCo assay
FVIII* assay	To assess FVIII level
RIPA assay	To assess sensitivity of ristocetin induced interaction of the patient's vWF with their own platelets
vWF multimer analysis	To assess the vWF multimer pattern by gel electrophoresis
Genetic analysis of vWF gene	To confirm a suspected type 2 vWD subtype

* normal range 50–150 iu/dl.
FVIII = factor VIII; PTT = partial thromboplastin time; RCo = ristocetin cofactor assay; RIPA = ristocetin induced platelet aggregation; vWF = von Willebrand factor.

static role of platelets, which explains the mucocutaneous bleeding characteristics of vWD.

Laboratory investigations of von Willebrand disease

These are listed in Table 1.

Classification of von Willebrand disease

The modern classification of vWD, formulated in 1994, has recently undergone some minor modifications.[4,5] The disease is classified into three main types, 1, 2 and 3, with type 2 divided into four subtypes (Table 2).[6]

Type 1

Type 1 vWD, the most prevalent form of vWD (about 70% of cases), is defined as a partial quantitative deficiency of vWF and has an autosomal dominant inheritance pattern. The main laboratory findings are similarly reduced vWF antigen and activity levels, normal or impaired ristocetin induced platelet aggregation (RIPA), with no increased sensitivity to ristocetin at low concentrations and a full set of multimers.

Only a relatively small number of causative mutations have been identified in the vW gene giving rise to a failure of vWF production, which in the heterozygous state would result in mildly reduced vWF levels as observed in type 1 vWD. In fact, most kindreds with type 1 vWD do not have an identifiable vWF gene mutation.[7] It is possible that in these kindreds there may be as yet unidentified causative mutations in relatively inaccessible parts of the vWF gene such as in the intron regions. It has been suggested that a significant number of cases may result from the influence of 'distant genes' on vWF levels and simply represent the lower extreme of normality. For example, ABO blood group has a significant effect on vWF levels, with group O individuals having up to 25% lower vWF levels than those with non-O because of their more rapid clearance of vWF from the circulation. Mildly reduced vWF levels are a poor predictor of a bleeding tendency; many patients found to have a type 1 vWD phenotype on laboratory testing do not bleed excessively. It is therefore important to take a detailed bleeding history in individuals with mildly reduced vWF levels to ensure that they are not falsely labelled as having a clinically significant bleeding disorder.

Type 2 vWD

Type 2 vWD, which accounts for around 25% of cases, is subdivided into four subtypes.

Type 2A

Genetic mutations either cause a failure of normal multimer formation (group 1 mutations) or impart increased sensitivity of multimers to degradation by the vWF cleaving protease ADAMTS13 in the circulation (group 2 mutations). The result in both groups is a loss of circulating high and intermediate molecular weight multimers, which explains the laboratory findings of a reduced vWF antigen level with a disproportionately lower ristocetin cofactor assay (RCo). The loss of the multimers is confirmed on multimer gel analysis.

Type 2B

Mutations in the region of the platelet glycoprotein (GP) Ib binding site result in increased affinity of vWF for platelets so that vWF multimers bind spontaneously to them in the circulation, causing loss of high molecular weight multimers from the plasma. As a consequence, similar to

Table 2. Classification of von Willebrand disease.

Type	Description
1	Partial quantitative deficiency of vWF
2A	– group 1 mutations (impaired multimer assembly) – group 2 mutations (increased proteolysis of vWF in circulation)
2B	Increased affinity of vWF for platelet GPIb receptor
2M	Decreased affinity of vWF for platelet GPIb receptor
2N	Decreased affinity of vWF for factor VIII
3	Complete quantitative deficiency of vWF

GP = glycoprotein; vWF = von Willebrand factor.

individuals with type 2A, patients have a reduced vWF antigen level with a disproportionately lower RCo level. Patients demonstrate increased RIPA sensitivity due to the prebound vWF on platelets, and the loss of high molecular weight multimers is confirmed on gel analysis. Some mutations are associated with the development of thrombocytopenia as a result of increased clearance of the vWF coated platelets.

Type 2M

Mutations in the region of the platelet GPIb binding site result in decreased affinity of vWF, reducing the ability of vWF multimers to interact with platelets. Again, patients have a reduced vWF antigen level with a disproportionately lower RCo and also demonstrate markedly reduced RIPA activity. Multimer analysis shows a full set of multimers.

Type 2N (vWD Normandy)

Mutations in the FVIII binding site lead to reduced affinity of vWF for FVIII. Type 2N is an autosomal recessive disorder as the phenotypic abnormality (reduced circulating FVIII levels) occurs only in homozygotes. FVIII levels in homozygotes can be in single figures whereas vWF antigen and activity levels, RIPA and multimer studies are normal. The diagnosis can be confirmed by performing vWF/FVIII binding studies and gene analysis.

Key Points

- **von Willebrand disease (vWD) is the most common of the inherited bleeding disorders**
- **Individuals with vWD have a mucocutaneous bleeding pattern**
- **vWD is caused by relative (type 1) or absolute (type 3) quantitative or qualitative (type 2) defects in the synthesis of von Willebrand factor (vWF)**
- **Type 2 vWD is subdivided into 2A, 2B, 2M and 2N**
- **Desmopressin is the treatment of choice for patients with type 1 vWD**
- **Patients with types 2 and 3 require treatment with a factor VIII concentrate containing vWF**

KEY WORDS: desmopressin, factor VIII concentrate, hereditary bleeding disorder, von Willebrand disease, von Willebrand factor

Type 3

Type 3 accounts for 5% of cases and is caused by the presence of two null vWF genes, either by homozygous or double heterozygous inheritance. There is no detectable vWF antigen or activity, RIPA activity is absent and no multimers are present. The FVIII level can be very low and, as a consequence, patients may have the bleeding manifestations of haemophilia superimposed on their mucocutaneous bleeding problems.

Management of von Willebrand disease (Table 3)

Type 1

Patients with type 1 vWD can be treated with the synthetic hormone preparation desmopressin which releases vWF from blood vessel endothelial cells resulting in a two to fivefold rise in vWF levels.[8,9] Historically, desmopressin has been administered by slow intravenous infusion but subcutaneous or intranasal preparations are now preferred. Single doses are usually sufficient to treat bleeding episodes or to cover minor procedures including dental extractions. For more major procedures, several daily doses of desmopressin may be required. The major side effect of desmopressin is fluid retention with possible hyponatraemia. Patients should therefore be fluid restricted; if several doses of desmopressin are required, fluid balance and electrolytes need to be closely monitored.

For patients who do not respond to or cannot tolerate desmopressin or in whom it is contraindicated (over-70s, cardiovascular disease) a FVIII clotting factor containing vWF should be administered.[10,11] Cryoprecipitate has previously been used to replenish vWF levels but is no longer considered because of the potential risk of transfusion-associated infection.

Types 2A, 2B, 2M and 3

Desmopressin is ineffective in raising vWF levels in individuals with type 2A with a group 1 mutation, types 2M and 3. A FVIII concentrate containing vWF is required for haemostasis. Individuals with type 2A group 2 mutations and type 2B tend to have good increases in vWF levels with desmopressin but the released vWF has a much shortened half-life due to increased clearance. Desmopressin may be effective in the treatment of acute bleeding in these individuals but for more sustained haemostasis (as in surgery) a FVIII concentrate containing vWF will be required. Some individuals with type 2B may develop significant thrombocytopenia with desmopressin and it is therefore contraindicated. The reduced FVIII levels in type 2N are corrected by the use of a FVIII concentrate containing vWF.

Table 3. Haemostatic treatment options in von Willebrand disease.

Type	Agent
1	Desmopressin or FVIII concentrate containing vWF
2A GPI mutations	FVIII concentrate containing vWF
2A GPII mutations	Desmopressin or FVIII concentrate containing vWF
2B	Desmopressin or FVIII concentrate containing vWF if thrombocytopenia develops with desmopressin
2M	FVIII concentrate containing vWF
2N	FVIII concentrate containing vWF
3	FVIII concentrate containing vWF

FVIII = factor VIII; GP = glycoprotein; vWF = von Willebrand factor.

References

1 Sadler JE, Mannucci PM, Berntorp E *et al.* Impact, diagnosis and treatment of von Willebrand disease. *Thromb Haemost* 2000; 84:160–74.
2 Sadler JE. Biochemistry and genetics of von Willebrand factor. Review. *Annu Rev Biochem* 1998;67:395–424.
3 Ruggeri ZM. Structure of von Willebrand factor and its function in platelet adhesion and thrombus formation. Review. *Best Pract Res Clin Haematol* 2001;14:257–79.
4 Sadler JE. A revised classification of von Willebrand disease. For the Subcommittee on von Willebrand Factor of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. *Thromb Haemost* 1994;71: 520–5.
5 Sadler JE, Budde U, Eikenboom JC *et al.* Update on the pathophysiology and classification of von Willebrand disease: a report of the Subcommittee on von Willebrand Factor. Review. *J Thromb Haemost* 2006;4:2103–14.
6 Laffan M, Brown SA, Collins PW *et al.* The diagnosis of von Willebrand disease: a guideline from the UK Haemophilia Centre Doctors' Organization. *Haemophilia* 2004; 10:199–217.
7 Cumming A, Grundy P, Keeney S *et al.* An investigation of the von Willebrand factor genotype in UK patients diagnosed to have type 1 von Willebrand disease. *Thromb Haemost* 2006;96:630–41.
8 Pasi KJ, Collins PW, Keeling DM *et al.* Management of von Willebrand disease: a guideline from the UK Haemophilia Centre Doctors' Organization. *Haemophilia* 2004;10:218–31.
9 Mannucci PM. Desmopressin (DDAVP) in the treatment of bleeding disorders: the first 20 years. Review. *Blood* 1997;90:2515–21.
10 Lillicrap D, Poon MC, Walker I *et al.* Efficacy and safety of the factor VIII/von Willebrand factor concentrate, haemate-P/humate-P:ristocetin cofactor unit dosing in patients with von Willebrand disease. *Thromb Haemost* 2002;87:224–30.
11 Mannucci PM, Chediak J, Hanna W *et al.* Treatment of von Willebrand disease with a high-purity factor VIII/von Willebrand factor concentrate: a prospective, multicenter study. *Blood* 2002;99:450–6.

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Molecularly targeted therapy in myeloid leukaemias

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Until recently it was difficult to detect the impact of recent advances made in the understanding of the biology of leukaemogenesis on the treatment of the myeloid leukaemias. However, in the past five years the therapeutic benefits which can flow from advances in basic biomedical research have been conclusively demonstrated by the remarkable clinical activity of imatinib, a small molecule inhibitor of the *BCR-ABL* tyrosine kinase fundamental to the pathogenesis of chronic myeloid leukaemia (CML). This has provided a model for the development of novel therapies in acute myeloid leukaemia (AML).

Targeted therapy in chronic myeloid leukaemia

CML is a myeloproliferative disease which typically presents with a high white count, splenomegaly and constitutional symptoms. Its natural history is to evolve within three to five years from a chronic phase (CP) into an advanced stage (accelerated phase or blastic transformation) which is usually rapidly fatal.[1] The presence in over 95% of patients of the Philadelphia (Ph) chromosome, characterised cytogenetically by the presence of a reciprocal translocation between chromosomes 9 and 22, led to the identification of the *BCR-ABL* fusion gene, the molecular hallmark of CML. The *BCR-ABL* oncogene is a constitutively activated tyrosine kinase with the capacity to transform haematopoietic cells *in vitro* and in mouse models through its ability to activate downstream pathways which confer increased proliferation, decreased growth factor independence and reduced apoptosis on haematopoietic progenitors. Consequently, *BCR-ABL* represents an attractive therapeutic target in CML.

Imatinib

Pioneering work in the 1990s by medicinal chemists identified imatinib mesylate as an oral inhibitor of *BCR-ABL* which acts through its ability to bind in the region of the ATP binding site of the BCR-ABL protein.[2] Imatinib is able to induce selective cytotoxicity of *BCR-ABL*-positive leukaemic cell lines; this has led to its rapid introduction into clinical practice where it has shown remarkable activity in patients with CML.

Earlier therapies

Treatment options in CML before the development of imatinib were limited to the long-term administration of interferon (IFN) alpha or allogeneic stem cell transplantation. IFN was in many ways a spectacularly ineffective therapy which combined toxicity with lack of activity in most patients. However, a minority of patients (ca 15%) achieved a complete cytogenetic remission (CCR), defined as the absence of Ph+ve metaphases in a bone marrow aspirate. Importantly, such patients demonstrated markedly improved survival, supporting the notion that elimination of the Ph+ clone from the bone marrow could be used as a surrogate marker of improved survival.[3] Allogeneic transplantation remains an important curative procedure in younger patients with an available sibling or unrelated donor, and is the only treatment with the proven ability to produce long-term disease-free survival in patients with CML.[4] The lack of activity of IFN alpha in most patients and the toxicity of transplantation meant that the great majority of patients with CML had no effective treatment option until the development of imatinib.

Treatment with imatinib

The demonstration in 1999 of the ability of imatinib to induce haematological and cytogenetic remissions in patients who had failed to respond to IFN alpha