In addition, the orientations of GPRVVEam in the 'a' and 'b' sites are essentially identical. When the structures of the peptide bound in these two sites are superimposed, the only notable difference is the orientation of the last discernable carboxylates, such that the peptides exit their respective sites in different paths. This superposition also shows that the two binding sites are remarkably similar. Because these chains likely evolved from a common ancestor [8], it is anticipated that they will have similar structures. Nevertheless, the similarity is remarkable because this region is composed of multiple loops and essentially no secondary structure.

Four calcium-binding sites, β1, β2, γ1, and γ2, have been identified in previous structures [4,9]. In the presence of GPRVVEam, calcium ions clearly occupy sites γ1 and β1 (Fig. 1A), which are juxtaposed to 'a' and 'b' sites, respectively. These sites are identical to those previously described in the structures of DDBOTH [5] and rfD-BOTH [4]. Calcium-binding site β2, which is co-ordinated to the side chains of residues γE132, βD261, βE397, and βD398 in the absence of peptide ligands [4,5], was unoccupied when GPRVVEam was bound to the 'b' site, as demonstrated by no significant |Fo − Fc| difference density contoured at 2.5 σ. As previously reported for rfD-BOTH, the side chains of two of these four residues, βE397 and βD398, interact with the bound peptide, and the side chain of a third, γD132, forms a salt link with αK157 [4]. As expected, the calcium-binding site γ2, reported in some structures when GHRPam is bound in the 'a' site [5], was not seen in this structure. In conclusion, these data demonstrate that a peptide that accurately mimics the N-terminus of the α-chain indeed binds in both the 'a' and 'b' sites within a single D fragment. It is reasonable, therefore, to postulate that 'A:b' interactions may participate in fibrin polymerization. Whether such interactions are transient, and replaced by 'B:b' interactions when the 'B' knob is exposed, or are retained as a significant factor in normal polymerization remains to be established.

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Factor VIII intron-1 inversion: frequency and inhibitor prevalence

J. SCHRÖDER,* O. EL-MAARRI,* R. SCHWAAB,* C. R. MÜLLER† and J. OLDENBURG*†
**Institute of Experimental Haematology and Transfusion Medicine, University of Bonn, Bonn, Germany; and †Institute of Human Genetics, University of Würzburg, Würzburg, Germany*

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Correspondence: Johannes Oldenburg, Institute of Experimental Haematology and Transfusion Medicine, Sigmund-Freud Str 25, 53127 Bonn, Germany.
Tel.: +49 228 287 5175/5176; fax: +49 228 287 4320;
e-mail: johannes.oldenburg@ukb.uni-bonn.de

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Hemophilia A (HA) is caused by reduced or absent clotting factor VIII (FVIII) activity because of a wide spectrum of mutations in the *FVIII* gene. The *FVIII* gene is located at the telomeric end of chromosome Xq28. Two long repeats are located within the *FVIII* locus: the Int22h-1 in intron 22 and the Int1h-1 in intron 1. The former is 9.1 kb in length and has

Table 1 Summarized data for prevalence of intron-1 inversion and development of inhibitor against factor VIII; the populations are listed by decreasing order of frequency of intron-1 inversion

Population/reference	Number of patients	intron-1 inversion	Prevalence (%)	Patients with inhibitor	Prevalence of inhibitor (%)
Spain [5]	79	4	5.06	0	0
Italy I [7]	20	1	5.0	0	0
England I [3]	209	10	4.78	1	10
Czech Republic [8]	162	7	4.32	1	14.29
India I [9]	80	3	3.8	0	0
Germany (this study)	1127	23	2.04	6	26.09
England II [10]	595	11	1.85	1	9.09
Italy II [4]	293	5	1.71	1	20
India II [11]	241	3	1.24	0	0
Hungary [6]	104	0	0	0	0
Total	2910	67	2.30	10	14.93

two additional homologues (Int22h-2 and 3) at about 500–580 kb distance towards the telomer. The latter is about 1 kb and has one homologue located 141 kb towards the telomere. Both repeats are prone to intrachromosomal homologous recombination that cause inversion of the intervening sequence leaving the *FVIII* gene splitted into two parts of opposite transcriptional direction [1,2]. The clinical result of such inversions is a severe HA phenotype with no functional FVIII protein.

In this study, we compared the incidence of the intron-1 inversion among different populations and investigated the correlation of this type of mutation with the development of inhibitors. Bagnall *et al.* [3] have designed a simple polymerase chain reaction (PCR) method to detect the intron-1 inversion. Previously, several studies have investigated the frequency of this inversion among hemophiliacs (Table 1). The observed frequencies ranged from 1.7% as reported by Salviato *et al.* [4] for the Italian population to 5.1% in the Spanish population (Tizzano *et al.* [5]). However, Andrikovics *et al.* [6] did not found the intron-1 inversion among 104 Hungarian severe HA patients. Using the PCR approach developed by Bagnall *et al.* [3] we analyzed 1127 patients with severe HA and could detect 23 patients with an intron-1 inversion (2.04% of the severe cases). These patients were sent by most hemophilia centers throughout Germany and comprise about half of the severe HA population of our country.

The striking variability in the incidence of the intron-1 inversions among different studies could be the result of several factors. The largest contribution to this observed variability could be the sample size of patients analyzed: The first five studies (Table 1) [3,5,7–9] represent a total of 550 patients with an incidence of 4.5% for the intron-1 inversion. Later studies (the last five studies in Table 1) [4,6,10,11, this study] included larger number of patients (2360 in total) and reported a lower incidence of 1.78% (Table 1). Another variable in these studies might be the inclusion of non-severe hemophiliacs because of misclassification because of the variability of residual FVIII levels. Furthermore, if intron-1 inversions were to cluster in some populations because of a higher proportion of distantly related patients this should inflate the incidence of this mutation. Differences in the rate of homologous recombination could also influence the frequency of the intron-1 inversion in a given population, however not much is known about such factors. In our cohort almost all of the centers applied the FVIII:C one-stage-assay and we are not aware of misclassified patients. None of the German centers showed a clustering of the intron-1 inversion mutation.

Both, the intron 1 and the intron 22 inversion have similar mutation mechanisms and both irreversibly disrupt the *FVIII* gene structure thus leading to abortive transcription and translation. Consequently, the prevalence of inhibitor formation should be comparable between both mutation types. For the intron 22 inversion, inhibitor prevalence between 21.5% and 34.5% have been reported [12,13]. In all previous studies on intron-1 inversion patients, the cumulative inhibitor prevalence is about 9.0% (4 of 44; Table 1). In our cohort, six patients out of 23 developed an inhibitor against the FVIII protein, with three high responders, two low responders and one patient of unknown inhibitor titer. Thus, the calculated inhibitor prevalence of 26.1% agrees well with that found among intron 22 inversion carriers (21.5–34.5%; [12,13]).

In summary, it seems that the frequency of the intron-1 inversion is lower and the risk of inhibitor development higher than reported in most previous studies. Nevertheless, this mutation remains the second most frequent molecular cause of HA after the intron 22 inversion. As it is easy and fast to analyze, we recommend this test as the second step in a mutation-screening program after exclusion of the intron 22 inversion and before screening of the entire gene.

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Clopidogrel resistance caused by a failure to metabolize clopidogrel into its metabolites

A. A. C. M. HEESTERMANS,* J. W. VAN WERKUM,* E. SCHÖMIG,† J. M. TEN BERG* and D. TAUBERT†

*Department of Cardiology, St Antonius Hospital, Nieuwegein, the Netherlands; and †Department of Pharmacology, University Hospital, University of Cologne, Cologne, Germany

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We report about a patient with acute stent thrombosis, unimpaired clopidogrel absorption but failure to metabolize clopidogrel. Although the mechanism of this failure remains speculative, this case links an inadequate clopidogrel response to clinical outcome. The combination of clopidogrel and aspirin has become standard therapy in patients undergoing percutaneous coronary intervention (PCI) to prevent stent thrombosis. Clopidogrel is a prodrug that is absorbed in the intestine. About 85% of the drug is hydrolyzed by esterases to an inactive carboxylic acid derivative. However, a small fraction is converted into the intermediate metabolite 2-oxo-clopidogrel by hepatic cytochrome P450 (CYP3A4) monooxygenase [1]. The instable 2-oxo-clopidogrel is non-enzymatically hydrolyzed to the active thiol metabolite that irreversibly binds to the adenosine diphosphate (ADP) P2Y12 receptor resulting in a partial inhibition of platelet aggregation [1]. However, several studies have demonstrated a considerable interindividual variability in the response to clopidogrel and Matetzky *et al*. [2] have recently demonstrated the clinical relevance of an inadequate platelet response to clopidogrel.

Correspondence: J. M. ten Berg, Department of Cardiology, St Antonius Hospital, PO Box 2500, 3435 CM, Nieuwegein, the Netherlands.
Tel.: +31 306099111; fax: +31 306034420; e-mail: berg03@antonius.net

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Case

A 38-year-old woman with no medical history was presented at our catheterization laboratory with an acute inferior myocardial infarction. The patient gave informed consent to obtain additional blood samples to investigate platelet response to a 600 mg loading dose of clopidogrel. Before catheterization clopidogrel 600 mg and aspirin 300 mg were administered orally and heparin 70 IU kg^{-1} was given intravenously. Coronary angiography of the right coronary artery showed a thrombotic occlusion with trombolysis in myocardial infarction (TIMI) I flow. The left coronary artery appeared to be normal. The thrombus was aspirated and two TAXUS® Express™ stents (3.5 × 32 mm and 3.5 × 24 mm) were placed resulting in TIMI III flow. A postprocedural electrocardiogram (ECG) showed resolution of ST segment elevation and the