Introduction

The amount of residual factor VIII (FVIII:C) determines the clinical variability of hemophilia A. About 50% of the patients have severe hemophilia A with a FVIII:C activity less than 1% of normal. Moderate (FVIII:C 2–5% of normal) and mild (FVIII:C > 5% of normal) hemophilia A occur in about 10% and 30–40% of patients, respectively. Recently we showed that the mutation detection rate in severely affected male patients is virtually 100% when testing for the common intron 22/-intron 1-inversions and big deletions, followed by genomic sequencing of the F8 gene. We also showed that protein truncating molecular defects are prevalent in those patients [1]. Here we report on the mutation detection rate and the spectrum of mutations in 136 patients with mild/moderate hemophilia A, as well as their distribution throughout the F8 protein.

Patients and Methods

Twenty four moderately and 112 mildly affected patients of German origin were included in the study. Genomic DNA was extracted from peripheral blood lymphocytes using salting procedure. The coding region of the F8 gene, the intron/exon boundaries and the F8 promoter region were subjected to sequencing analysis using primers and conditions as previously described [1]. The newly detected amino acid substitutions were scored for potential gross or local conformational changes and influence on molecular stability for every single F8 domain with available structures, using homology modeling [2–4].

Results and Discussion

Mutation Detection Rate

The performed sequencing analysis revealed a molecular defect in 121 (89%) of the patients, whereas 15 (11%) had no mutation in the coding region of the F8 gene, in the exon/intron borders or in the promoter region. All negative patients were mildly affected with exception of one patient with FVIII:C 2–3%.
Spectrum and Distribution of the Mutations

In the group of moderate hemophilia A patients 22 (91.6%) were affected by missense mutations. Four of these changes are novel: G236A, L172F (A1 domain), G236A (A2 domain) and A1779S (A3 domain). One patient (4.2%) had a small deletion of an adenine in an A-stretch in exon 14 of the F8 gene and in another one (4.2%) no mutation could be detected on genomic level.

The vast majority (83/112, 74%) of mutations detected in the group of mildly affected patients were also missense. Twenty seven of these molecular defects are described for the first time, namely in the A1 domain: G244D, V178G, T262P, I76S, I173T, D163A and V101D; in the A1/A2 boundary V357G; in the A2 domain: G563A, I548F, C630R, T657S and P526H; in the A2/B boundary C692R and T751S; in the A3 domain Y1815C, P1825H, M2010I, E2004K and V1873G; in the C1 domain R2150L, F2126S, T2154N, R2150L and S2133P and in the C2 domain G2325A and P2292H. Nine (8%) patients were shown to carry a splicing mutation and two – a deletion/insertion of an A in a poly-adenosin stretch in the B-domain. Three of the splicing errors are novel alterations, these are: D318Y/splice (A1 domain), IVS15-1 G>A (A3 domain) and IVS21 +1G>T (C1 domain).

The molecular defects are systematically presented in Figure 1. They are distributed throughout the whole sequence of the factor VIII gene, but predominantly in the A2 (mainly exon 11) and C1 (mainly exon 23) domains.

The novel amino-acid substitutions were scored for potential conformational changes and influence on the molecular stability, using homology modeling. Figure 2 illustrates the effects of the conservative mutation P1825H (A3 domain), detected in a mildly affected patient (slight change of loop conformation) as compared to P1825S previously published in a moderate affected patient (severe distortion of loop conformation).

Two molecular changes (−112G>A and −219C>T) in the promoter region of the factor VIII gene were detected in two patients with mild hemophilia A. These defects were excluded in a cohort of 100 normal males and have not been detected in more than 300 hemophilia A patients with known mutations. Both changes affect important promoter sites, thus supporting possible pathogenicity of these alterations. To our knowledge this is the first report on promoter mutations in the F8 gene.

Conclusion

Our data show that, in contrast to severe hemophilia A, the analysis on genomic level fails to detect the molecular defect in about 4% of the moderately and in 12.5% of the mildly affected patients. While the mutation detection rate in moderate hemophilia A is still high (<95%), it is significantly lower in the mildly affected cases (87.5%), thus suggesting that the reduction of the F8 activity could be due to other (probably unknown) reasons at least in a part of these patients.
Fig. 1. Spectrum and distribution of the mutations in the F8 gene, identified in 24 moderately and 112 mildly affected hemophilia A male patients.
Fig. 2. Dreiding model images of a part of the F8 A3 domain around P1825 (left panel) with the mutation P1825H (middle panel) and with the mutation P1825S (right panel). Protein chains are colored in light blue, selected amino acids are dark blue. Disulfide bonds are presented in yellow, hydrogen bonds in red and ionic bonds in magenta. Residues participating in ionic bonds are highlighted in green (where not selected in dark blue).

References