Factor XII Osaka: Abnormal factor XII with partially defective prekallikrein cleavage activity

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Summary
A healthy Japanese male had reduced factor XII (FXII) activity (35%) in contrast to normal antigen levels (81%). The F12 of this proband had a 9775G to C mutation in exon 10 and an 11276G to A mutation in exon 13 that resulted in two amino acid substitutions of Ala324Pro (GGC→CCG) in the proline-rich connecting region and Gly531Glu (GGG→GAG) near the active Ser544 in the catalytic domain. His father had the nucleotide 46T/T and a heterozygous 9775G/C mutation. The FXII activity (32%) and antigen level (38%) of the father were about half that of normal individuals with 46T/T, suggesting a heterozygous cross reacting material (CRM)-negative deficiency. His mother had a 46C/T and heterozygous 11276G/A mutation, and 80% FXII activity was incompatible with the corresponding antigen level (125%), suggesting a heterozygous CRM-positive deficiency. The substitution of Ala324Pro probably caused the CRM-negative mutation and the Gly531Glu caused the CRM-positive mutation. We developed three methods based on chromogenic substrates to assay the distinct functions of FXII, namely its autoactivation on a negatively charged surface, activation by kallikrein cleavage and the prekallikrein cleavage activity of FXIIa. The ratios of autoactivated FXIIa/FXII antigen (0.80–1.10) and of kallikrein-induced FXIIa/FXII antigen (0.86–1.00) in plasma from the proband were within normal ranges, whereas those of FXIIa-induced kallikrein/FXII antigen were reduced to 0.41–0.45. In conclusion, the 9775G to C and 11276G to A mutations of F12 led to a CRM-negative and -positive FXII deficiency, and the F12 with 11276A produced a dysfunctional type of FXII with a partial defect (0.41–0.45) in prekallikrein cleavage activity.

Keywords
Amino acid substitution, catalytic domain, chromogenic substrate, F12 haplotype, intrinsic coagulation pathway

Introduction
Human coagulation factor XII (FXII) is a serine protease protein precursor that is primarily produced by hepatocytes and secreted in circulating plasma as a mature protein composed of 596 amino acid residues (1). FXII plays a central role in initiating the intrinsic pathway of blood coagulation. It binds upon contact to negatively charged surfaces in vitro, such as glass, kaolin, dextran sulfate, sulfatide and ellagic acid, and it is autoactivated to the active form (FXIIa) (2), which then proteolyses plasma prekallikrein to kallikrein. Kallikrein reciprocally activates more FXII (3), which initiates the rapid intrinsic coagulation pathway through FXI proteolysis to FXIa.

Recent investigations (4, 5) have demonstrated two physiological functions of FXII, one arising from the zymogen binding to endothelial cells and another for part of an injury response arising in disease states. FXII and FXIIa bind to urokinase-type plasminogen activator receptor on endothelial cells to stimulate cell growth and proliferation leading to angiogenesis. In the presence of flowing blood, FXII binds and autoactivates on exposed tissue RNA, polymers from activated platelets, aggregated and misfolded protein, or collagen exposed on injured endothelium. FXII autoactivation at sites of vessel injury initiates the contact activation of blood coagulation with the formation of thrombin. These events contribute to the extent of thrombus formation in vivo (4).

The human FXII gene (F12) comprises 14 exons and 13 introns and it is located on the chromosomal band 5q33-qter (5). The protein structure of FXII is composed of one light and one heavy chain. The heavy chain consists of fibronectin type I and II domains, two epidermal growth factor domains, a kringle and a proline-rich connecting domain, whereas the light chain consists of a catalytic domain.

Several investigators have described the molecular basis of a congenital FXII deficiency (6–8), which usually results in a lack of immunologically detectable FXII protein. This state is referred to as being cross-reactive material (CRM)-negative deficient. In contrast, only four individuals with immunologically identifiable FXII protein have been described as having a CRM-positive FXII deficiency (9–11).
We describe here a compound heterozygote with a CRM-negative and CRM-positive FXII deficiency and novel FXII amino acid substitutions of Ala324Pro and Gly531Glu. The plasma of the proband contained FXII derived from a mutant F12 with 11276A (Glu531) that had a partial defect in prekallikrein cleavage activity. We refer to this protein as Factor XII Osaka. This is the first report of a CRM-positive FXII deficiency with partially impaired FXII activity.

Materials and methods

Blood collection

The proband and his family provided written, informed consent to the collection of blood samples and to analyse their F12 in this study, which was approved by the Ethics Committee of the Faculty of Medicine, Tottori University. Normal pooled plasma was collected from 40 healthy Japanese volunteers.

Coagulation studies

Activated partial thromboplastin time (APTT) was measured using an APTT reagent (TriniCLOT aPTT HS; Trinity Biotech Plc., Bray, Ireland).

Specific coagulation factor activities were measured based on the APTT method using specific factor-deficient plasma (George King Bio-Medical, Overland, KS, USA) as substrate.

Factor XII antigen levels were determined by single radial immunodiffusion using anti-FXII antibody (Acris Antibodies GmbH, Herford, Germany).

Assay of FXIIa functions

Procedure I. Autoactivation of FXII on a negatively charged surface.

- Step 1. Normal pooled and FXII-deficient plasma, as well as plasma samples from the proband and his family were diluted 1:50 (vol/vol) in imidazole buffer (50 mM imidazole containing 100 mM NaCl, pH 7.3). Standards comprised various proportions of 1:50 diluted normal pooled and FXII-deficient plasma samples.
- Step 2. Standard or proband/family plasma samples (100 μl) diluted in step 1 were incubated with 10 μl each of EDTA (30 mM) and 1:10 diluted anti-plasma prekallikrein antibody (Acris Antibodies GmbH, Herford, Germany) at 37°C for 3 minutes (min), followed by the addition of 10 μl of APTT reagent (Thrombocheck APTT-SLA, Sysmex Co., Kobe, Japan) and incubation at 37°C for 60 min.
- Step 3. Chromogenic substrate (100 μl; 2 mM S-2302, Chromogenix AB, Stockholm, Sweden) was added.

Polymerase chain reaction (PCR) and DNA sequencing

Genomic DNA was prepared from peripheral blood leukocytes. Amplification of F12 was performed using PCR with oligonucleotide primers designed to cover all included exons and intron-exon junctions (12). Cycle sequencing was performed using the BigDye® Terminator Sequencing kit (Applied Biosystems, Inc., Foster City, CA, USA).
Table 1: Coagulation studies and FXII functions of proband and his family. *Estimated specific FXII act/antigen and †specific kallikrein/FXII antigen levels for mutant FXII molecule in mother’s plasma. ‡Normal ranges differ from types of nucleotide 46C/C, C/T or T/T.

<table>
<thead>
<tr>
<th></th>
<th>Proband 1st</th>
<th>Proband 2nd</th>
<th>Proband 3rd</th>
<th>Father</th>
<th>Mother</th>
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<td>58.5</td>
<td>63.2</td>
<td>58.7</td>
<td>45.7</td>
<td>40.9</td>
<td>42.1</td>
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<tr>
<td>FXII activity (%)</td>
<td>35</td>
<td>31</td>
<td>38</td>
<td>29</td>
<td>80</td>
<td>66</td>
</tr>
<tr>
<td>FXII antigen level (%)</td>
<td>81</td>
<td>75</td>
<td>73</td>
<td>30</td>
<td>125</td>
<td>66</td>
</tr>
<tr>
<td>FXII act/antigen</td>
<td>0.43</td>
<td>0.41</td>
<td>0.52</td>
<td>0.97</td>
<td>0.64</td>
<td>0.52*</td>
</tr>
<tr>
<td>Autoactivated FXIIa (%) (Generated FXIIa/FXII antigen)</td>
<td>65 (0.80)</td>
<td>83 (1.10)</td>
<td>68 (0.93)</td>
<td>31 (1.03)</td>
<td>154 (1.23)</td>
<td>60 (1.23)</td>
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<tr>
<td>Kallikrein-induced FXIIa (%) (Generated FXIIa/FXII antigen)</td>
<td>70 (0.86)</td>
<td>80 (0.94)</td>
<td>73 (1.00)</td>
<td>31 (0.97)</td>
<td>110 (0.88)</td>
<td>66 (1.00)</td>
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<tr>
<td>FXIIa-induced kallikrein (%) (Generated kallikrein/ FXII antigen)</td>
<td>33 (0.41)</td>
<td>34 (0.45)</td>
<td>30 (0.41)</td>
<td>27 (0.90)</td>
<td>72 (0.58)</td>
<td>76 (1.15)</td>
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Table 1 summarises the laboratory findings of his parents and brother.

Assay of FXII functions

Autoactivation of FXII on a negatively charged surface

The hydrolysis of S-2302 correlated with FXII concentrations in standard plasma samples in step 2 of procedure I (Fig. 1). Precise data for procedure I were generated at 40% and 100% ratios of FXII sample concentrations. The coefficients of variation were 10.2% and 9.6% for intra-assay reproducibility, and 8.8% and 9.5% for inter-assay reproducibility (n = 10). Levels of FXIIa generated in the proband plasma were 55%, 58% and 63% and those in plasma from his father, mother and brother were 31%, 154% and 60%, respectively. These results are means of three analyses of each sample. The ratios of autoactivated FXIIa/FXII antigen in proband plasma were 0.80, 1.10 and 0.93, whereas those in plasma from his father, mother and brother were 1.03, 1.23 and 0.91, respectively (Table 1).

Activation of FXII induced by kallikrein cleavage

The hydrolysis of S-2302 correlated with concentrations of FXII in standard plasma samples in step 2 of procedure II (Fig. 1). Precise data for procedure II were generated at 40% and 100% ratios of FXII samples concentrations. The coefficients of variation were 6.8% and 5.6% for intra-assay reproducibility, and 5.3% and 5.2% for inter-assay reproducibility (n = 10). Levels of FXIIa generated in the proband plasma were 70%, 80% and 73%, whereas those in plasma from his father, mother and brother were 31%, 110% and 66%, respectively. These results are means of three analyses of each sample. The ratios of FXIIa/FXII antigen in proband plasma were 0.86, 1.07 and 1.00 and those in plasma from his father, mother and brother were 1.03, 0.88 and 1.00, respectively (Table 1).

Results

Case report and laboratory data

The proband was a 23-year-old male Japanese student with no history of abnormal bleeding or thromboembolism, in whom prolonged APTT of 58.5 seconds was identified during practical training for blood coagulation tests. His haemostatic laboratory findings were as follows: normal prothrombin time, 1.04 INR (International Normalised Ratio); FVIII activity, 91%; FIX activity, 160%; FXI activity, 100%; FXII activity, 35%; FXII antigen level, 81% and plasma prekallikrein activity, 140%.

For details see methods.
Prekallikrein cleavage activity of FXIIa

The hydrolysis of S-2302 correlated with concentrations of FXII in standard plasma samples in step 1 of procedure III (Fig. 1). Precise data for procedure III were generated at 40% and 100% ratio of FXII sample concentrations. The coefficients of variation were 7.2% and 5.2% for intra-assay reproducibility, and 6.8% and 7.8% for inter-assay reproducibility (n = 10). The levels of kallikrein in plasma from proband were 0.40, 0.45 and 0.41, whereas those in plasma from his father, mother and brother were 0.98, 0.58 and 1.15, respectively (Table 1).

Genetic analysis

Sequence analysis of F12 revealed that the proband was heterozygous for C and T at nucleotide 46 in the 5'-untranslated region near the initiation codon of exon 10 and had two distinct mutations. One was a 9775G to C mutation at codon 324 in exon 10, which resulted in an amino acid substitution of Ala (GCG) to Pro (CCG) for 46T/46C subjected to FXII cleavage activity. Another was a 11276G to A mutation at codon 531 in exon 13 that produced a Gly (GGG) to Glu (GAG) substitution for 46T/46C subjected to FXII cleavage activity (Fig. 2A). Another was a 11276G to A mutation at codon 531 in exon 13 that produced a Gly (GGG) to Glu (GAG) substitution for 46T/46C subjected to FXII cleavage activity (Fig. 2B). The father of the proband was homozygous for 46T and had a heterozygous substitution of Ala324Pro. The proband’s father was heterozygous for 46C/T and had a heterozygous substitution of Gly531Glu. The proband’s brother was homozygous for 46T and the sequences of codons 324 and 531 were normal.

Discussion

The proband was a novel compound heterozygote for Ala324Pro and Gly531Glu substitutions. Codon 324 is located in the proline-rich connecting region and codon 531 is located near the active serine544 in the catalytic domain (5).

The proband’s father had 46T/T, heterozygous 9775G/C and normal 11276G. His F12 haplotypes apparently consisted of a normal set of 46T-9775G-11276G and a mutant set of 46T-9775C-11276G. The proband’s brother had 46T/T, normal 9775G and normal 11276G. His F12 haplotypes apparently consisted of a normal set of 46T-9775G-11276G derived from his father and a normal set of 46T-9775C-11276G derived from his mother, who had 46T/C, normal 9775G and heterozygous 11276G/A. Because one haplotype of his mother’s F12 consisted of a normal set of 46T-9775G-11276G like his brother, the other haplotype should be a mutant set of 46C-9775G-11276A. The proband had 46T/C, 9775G/C and 11276G/A. One haplotype of his F12 appeared to comprise mutant sets of 46T-9775C-11276G and of 46C-9775G-11276A derived from his father and mother, respectively.

The mean levels of activity and antigen of normal Japanese individuals with 46T/T are 82 ± 19% and 61 ± 11% (12). The FXII activity (32%) and antigen level (38%) in plasma from the father were about half those of normal individuals with 46T/T. The normal haplotype consisting of 46T-9775G-11276G from the F12 of the father probably expresses normal FXII protein, whereas the mutant haplotype comprising 46T-9775C-11276G cannot, and the father is a heterozygote for a CRM-negative FXII deficiency caused by a 9775G to C mutation (Ala324Pro).

The FXII antigen levels of normal individuals with 46T/T and 46C/C are 61% and 178%, respectively (13). This suggests that a haplotype of 46T with 46C expresses FXII protein at a ratio of 1:3 compared to that expressed by a haplotype with 46C. The mother was heterozygous 46C/T and had 80% activity and an antigen level of 125%. Approximately 31% and 94% of the FXII antigen in plasma of the mother is apparently produced by the normal 45T-9775G-11276G and the mutant 46C-9775G-11276A haplotypes of the mother’s F12, respectively. The normal FXII protein of the mother (31%) accounts for 31% of the activity, and the remaining antigen (94%) accounts for the remaining activity (49%). Thus, the FXII protein derived from the mutant 46C-9775G-11276A haplotype is partially dysfunctional, with an efficacy of 0.52 (49%/94%).

The proband is a compound heterozygote of a CRM-negative mutation derived from his father and a CRM-positive mutation derived from his mother.
derived from his mother. The mutant 46T-9775C-11276G haplotype of the proband F12 derived from his father can not express FXII protein; thus, all FXII antigen in the proband’s plasma is derived from the remaining mutant 46C-977G-11276A haplotype. The antigen levels of this FXII protein conflicted with the activity levels, which were 0.43 (35%/81%), 0.41 (31%/75%) and 0.52 (38%/73%) in term of effectiveness. The estimated efficacy for the mother accorded with those for the proband. We designated this partially dysfunctional FXII as Factor XII Osaka.

The type of molecular substitution has not been provided for five of the nine described CRM-positive patients with a deficiency of FXII (14–17). Factor XII Washington D.C. is dysfunctional due to a Cys571Ser substitution in the catalytic domain, but the nucleotide mutation was not investigated (9). Factor XII Locarno has an amino acid substitution of Arg535 to Pro, which causes the loss of a kallikrein cleavage site (Arg353/Val354) [18]. Two CRM-positive FXII deficiencies (Asp442Asn and Gly570Arg) have been described by Schloesser et al. (11). The substitution of Asp442 to Asn (GAC→AAC) in the catalytic triad directly affects the catalytic activity. Gly570Arg (GCG→CGC) is located next to Cys571, which forms a vital disulfide bridge. Factor XII Osaka is a novel CRM-positive FXII deficiency caused by an amino acid substitution of Gly531 (GGG) to Glu (GAG) near the active serine 544 in the catalytic domain.

The autoactivation of FXII on a negatively charged surface, FXII activation induced by kallikrein cleavage and the proteolysis of prekallikrein and FXI by FXIIa are indispensable for the clotting activity of FXII. We developed three methods with which to measure the three distinct functions of FXII using the modified chromogenic substrate assay described by Hoem et al. (19).

Procedure I examined the autoactivation of FXII independently of kallikrein. Prekallikrein in standard plasma and proband/family plasma samples were neutralised by adding anti-plasma prekallikrein antibody in step 2. FXII autoactivated to FXIIa on a negatively charged surface in APTT reagent independently of kallikrein in step 3. Subsequently, FXIIa was neutralised by anti-plasma prekallikrein antibody in step 2. FXII autoactivated to FXIIa on a negatively charged surface in APTT reagent independently of kallikrein in step 3. Subsequently, FXIIa was neutralised by anti-plasma prekallikrein antibody. FXIIa hydrolysed the chromogenic substrate S-2302 in step 4. The ratios of generate FXIIa/antigen generated by the mutant molecule in prekallikrein cleavage activity.

In conclusion, we studied a healthy Japanese student with reduced FXII activity (35%) that contrasted with a normal antigen level (81%). He was a novel compound heterozygote of a CRM-negative (Ala324Pro) and CRM-positive (Gly531Glu) FXII deficiency. Mutated FXII protein (531Glu) was partially defective (0.41–0.45) in prekallikrein cleavage activity.

What does this paper add?

- We studied a healthy Japanese student with reduced FXII activity (35%) that contrasted with a normal antigen level (81%).
- He was a novel compound heterozygote of a CRM-negative (Ala324Pro) and CRM-positive (Gly531Glu) FXII deficiency.
- Mutated FXII protein (531Glu) was partially defective (0.41–0.45) in prekallikrein cleavage activity.

References