Short Report

Population-specific spectrum of the F11 mutations in Koreans: evidence for a founder effect


The aim of this study was to investigate a mutation spectrum of F11 among Korean patients with factor XI (FXI) deficiency and to determine the haplotypes of mutations frequently found in Koreans. Thirteen unrelated patients from non-consanguineous families with FXI deficiency were included in the study. In the mutation analysis, the most frequently found mutations were Q263X (four cases; 31%) and Q226X (three cases; 23%). The frequency of Q263X-bearing haplotype was significantly different between normal and patient groups (p = 0.001), which is consistent with a founder effect of Q263X mutation. Testing for the presence of these two mutations should be the first genetic screening in Korean patients with FXI deficiency.

Conflict of interest
Nothing to declare.

Factor XI (FXI) is a zymogen that contributes to hemostasis by activating factor IX (1). FXI deficiency (MIM#264900), also called hemophilia C, is a rare autosomal recessive bleeding disorder which is particularly common among Ashkenazi Jews (2). The common modes of presentation of FXI deficiency are bleeding following injury, including dental procedures, tonsillectomy, nose surgery, urologic procedures, and an incidental finding of prolonged activated partial thromboplastin time (APTT) during routine physical checkup or pre-operative laboratory workup (3). In patients with FXI deficiency, mutations were found in F11 (GenBank accession number, NM_000128.3) which is located on chromosome 4 (4q35) and is 23 kb in length with 15 exons and 14 introns (4). According to an F11 mutation database at http://www.factorxi.com, more than 191 disease-causing mutations in F11 (5) have been reported.

Most patients with severe FXI deficiency (FXI level < 20%) are found to harbor a homozygote mutation or compound heterozygote, while heterozygote individuals show milder presentation or show no symptoms (3).

Notably, while FXI-deficient patients in the Ashkenazi Jewish population commonly present the E117X and F283L mutations, non-Jewish patients with the same deficiency are found with different mutations. In Basques, C38R was found to be the predominant mutation (6), whereas C128X and Q88X were more frequently observed in Caucasians from the United Kingdom and families living in west France, respectively (7, 8). Interestingly, while the E117X and F283L mutations are frequently observed in the Jewish population and some European populations, they have never been found in Asians, suggesting possible existence of founder mutation. Although
specific population studies have not been carried out in Asians, two mutations (Q226X and Q263X) have been frequently reported in Japanese and Chinese patients (9–13).

In this study, we studied 13 unrelated Korean patients diagnosed with FXI deficiency to analyze the spectrum of F11 mutations. In addition, haplotype analysis was carried out to determine a common genetic background of a specific mutation, and to investigate a possible founder effect in the Korean population.

**Patients and methods**

**Patients**

Patients were selected based on the FXI level (<60% in normal plasma) and prolonged APTT. The clinical histories and laboratory results of the patients are summarized in Table 1. We selected a total of 13 patients and 50 healthy controls among individuals who visited our hospital for physical check up. The controls were screened for the prevalence of the two frequently found nonsense mutations and all novel mutations found in this study. Before the study began, informed written consent was collected from all participants according to the ethical guidance of the Institutional Review Board. For patients who were under the age of 14, informed consent was obtained from their parents.

**Laboratory assessment**

Citrated blood was used for coagulation factor assays which examined the levels of factors II through XII, prothrombin time (PT), and APTT which were measured using an automated coagulation analyzer (STA-R Evolution®, Diagnostica Stago, Asnieres, France). Other coagulation parameters, including lupus anticoagulant and fibrinogen, were also measured to rule out a possible secondary FXI deficiency. FXI antigen was measured by an AssayMax Human Factor XI enzyme-linked immunosorbent assay kit based on a biotynylated polyclonal antibody against FXI recognized by a streptavidin–peroxidase conjugate (Gentaur, Kampenhout, Belgium). FXI antigen level was measured in two patients who were heterozygote for novel missense mutations. The reference range was determined by measuring FXI antigen levels in 30 individuals with normal APTT and PT.

**DNA extraction, PCR amplification, and direct sequencing**

DNA was extracted from ethylenediaminetetraacetic acid whole blood samples with an Easy-DNA™ Kit (Invitrogen Corporation, Carlsbad, CA). The concentration and quality of genomic DNA was evaluated by Nanodrop (ND-1000, Thermo Scientific, Wilmington, DE). We designed primers (Table S1) to amplify 15 exons and flanking introns of F11 based on the sequences provided by Zucker et al. (14). The modified primer sequences are available upon request.

One hundred haploid genomes from unrelated control individuals were also analyzed by direct sequencing of the exons bearing the novel missense mutations to confirm that control individuals did not have the mutations. Polymerase chain reaction (PCR) was performed on 100 ng of genomic DNA using an AccuPower™ Premix (Bioneer, Daejeon, Korea) under the following amplification conditions: 94°C for 3 min followed by 50 cycles of 94°C for 1 min, 62°C for 10 s and 72°C for 15 s, and final extension at 72°C for 15 min. The PCR products were then purified using a QIAquick Gel Extraction Kit (Qiagen, Düsseldorf, Germany) and directly sequenced using a cycle method with the same primers for PCR and a Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) with following conditions: 96°C for 5 min followed by 24 cycles of 96°C for 10 s, 50°C for 5 s and 60°C for 4 min, and final extension at 72°C for 5 min, in conjunction with an ABI Prism 310 automated genetic analyzer (Applied Biosystems). To detect any sequence variation, the sequences were compared to the reference sequences using SEQUENCHER software (Gene Codes Corporation, Ann Arbor, MI).

**Bioinformatics study**

In order to predict functional effects of novel missense variations, we compared inter-species amino acid conservation using CLUSTALW (15) and assessed *in silico* prediction using protein function predicting software such as SIFT (16), POLYPHEN (17), and PMUT (18) based on the information obtained from the Uniprot (http://www.ebi.uniprot.org/; Swiss-prot code/Uniprot number: P03951).

**Haplotype analysis**

Haplotypes were determined by analyzing 10 polymorphic markers of *F11* (Table 2) in DNA samples obtained from 24 normal individuals (48 chromosomes) and 13 patients (26 chromosomes). The oligonucleotide primers used in *F11* haplotype analysis are available upon request. In addition to polymorphisms −231C>T, −138A>C,
<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Reason for referral</th>
<th>FXI:C (%)</th>
<th>FXI:Ag (ng/ml)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Zygosity</th>
<th>Codon (HGVS nomenclature)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Amino acid change&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Exon</th>
<th>Geographic origin</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>74</td>
<td>M</td>
<td>Bleeding during urologic procedure</td>
<td>4</td>
<td>—</td>
<td>Homo</td>
<td>c.841C&gt;T (Q263X)</td>
<td>p.Gln263X (Q263X)</td>
<td>8</td>
<td>Japanese, Chinese</td>
<td>(9, 10, 12, 13)</td>
</tr>
<tr>
<td>2</td>
<td>51</td>
<td>F</td>
<td>History of post-operative bleeding</td>
<td>2</td>
<td>—</td>
<td>Homo</td>
<td>c.841C&gt;T (Q263X)</td>
<td>p.Gln263X (Q263X)</td>
<td>8</td>
<td>Japanese, Chinese</td>
<td>(9, 10, 12, 13)</td>
</tr>
<tr>
<td>3</td>
<td>46</td>
<td>M</td>
<td>Pre-operative screening</td>
<td>5</td>
<td>—</td>
<td>Homo</td>
<td>c.841C&gt;T (Q263X)</td>
<td>p.Gln263X (Q263X)</td>
<td>8</td>
<td>Japanese, Chinese</td>
<td>(9, 10, 12, 13)</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>F</td>
<td>Pre-operative screening</td>
<td>53</td>
<td>7.1</td>
<td>Hetero</td>
<td>c.94G&gt;A (G14R)</td>
<td>p.Gly14Arg (G14R)</td>
<td>3</td>
<td>Novel</td>
<td>Present study</td>
</tr>
<tr>
<td>5</td>
<td>25</td>
<td>M</td>
<td>Pre-operative screening</td>
<td>3</td>
<td>—</td>
<td>Comp</td>
<td>c.730C&gt;T (Q263X)</td>
<td>p.Gln263X (Q263X)</td>
<td>7</td>
<td>Japanese</td>
<td>(11)</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>M</td>
<td>Health check up</td>
<td>48</td>
<td>—</td>
<td>Hetero</td>
<td>c.688T&gt;C</td>
<td>p.O312Arg (C212R)</td>
<td>7</td>
<td>NA</td>
<td>Present study</td>
</tr>
<tr>
<td>7</td>
<td>33</td>
<td>F</td>
<td>Pre-operative screening</td>
<td>38</td>
<td>2.6</td>
<td>Hetero</td>
<td>c.159C&gt;A (H35Q)</td>
<td>p.His35Gln (H35Q)</td>
<td>3</td>
<td>Novel</td>
<td>Present study</td>
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<tr>
<td>9</td>
<td>7</td>
<td>F</td>
<td>Pre-operative screening</td>
<td>39</td>
<td>—</td>
<td>Hetero</td>
<td>c.1201T&gt;C</td>
<td>p.Trp383Arg (W383R)</td>
<td>11</td>
<td>Novel</td>
<td>Present study</td>
</tr>
<tr>
<td>10</td>
<td>7</td>
<td>M</td>
<td>Epistaxis</td>
<td>12</td>
<td>—</td>
<td>Comp</td>
<td>c.730C&gt;T</td>
<td>p.Gln263X (Q263X)</td>
<td>7</td>
<td>Japanese</td>
<td>(11)</td>
</tr>
<tr>
<td>11</td>
<td>68</td>
<td>F</td>
<td>Pre-operative screening</td>
<td>2</td>
<td>—</td>
<td>Hetero</td>
<td>c.730C&gt;T</td>
<td>p.Gln263X (Q263X)</td>
<td>7</td>
<td>Japanese</td>
<td>(11)</td>
</tr>
<tr>
<td>12</td>
<td>22</td>
<td>M</td>
<td>Difficulty in bleeding control during nose surgery</td>
<td>20</td>
<td>—</td>
<td>Hetero</td>
<td>c.1253G&gt;T</td>
<td>p.Gly400Val (G400V)</td>
<td>11</td>
<td>Italian (Czech), Chinese, Japanese</td>
<td>(11, 22)</td>
</tr>
<tr>
<td>13</td>
<td>22</td>
<td>F</td>
<td>Difficulty in bleeding control during plastic surgery</td>
<td>&lt;1</td>
<td>—</td>
<td>Hetero</td>
<td>c.841C&gt;T</td>
<td>p.Gln263X (Q263X)</td>
<td>8</td>
<td>Japanese, Chinese</td>
<td>(9, 10, 12, 13)</td>
</tr>
</tbody>
</table>

Comp Hetero, compound heterozygote; Hetero, heterozygote; HGVS, Human Genome Variation Society; Homo, homozygote; F, female; FXI, factor XI; M, male; NA, not available.

<sup>a</sup>Reference range 7.3–17.5 ng/ml.

<sup>b</sup>The approved HGVS nomenclature with 'A' of the translation initiation codon ATG numbered as +1 was used to describe nucleotide numbering for coding level.

<sup>c</sup>Conventional numbering according to Asakai et al. (26) at the protein level omitting signal peptide counting start codon ATG as −18.
Population-specific spectrum of the \textit{F11} mutations in Koreans

Table 2. \textit{F11} haplotype of patients with factor XI deficiency and two common mutations observed in Koreans

<table>
<thead>
<tr>
<th>Marker name (^a)</th>
<th>Location</th>
<th>RS numbers</th>
<th>Position (bp)</th>
<th>Jewish or Italian haplotype</th>
<th>Cases 1/2/3</th>
<th>Case 5</th>
<th>Case 10</th>
<th>Case 11</th>
<th>Case 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>−231C&gt;T (^b)</td>
<td>Intron A</td>
<td>rs4253398</td>
<td>187425055</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>−196T&gt;G (^b)</td>
<td>Intron A</td>
<td>rs4253399</td>
<td>187425088</td>
<td>−</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>G</td>
<td>T</td>
</tr>
<tr>
<td>−138A&gt;C (^b)</td>
<td>Intron A</td>
<td>rs3822057</td>
<td>187425146</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>(CA)n</td>
<td>Intron A</td>
<td>rs4253413</td>
<td>187433504</td>
<td>G</td>
<td>G</td>
<td>G</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>−431G&gt;A (^b)</td>
<td>Intron E</td>
<td>rs2055916</td>
<td>187433574</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>T</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>−361C&gt;T (^b)</td>
<td>Intron E</td>
<td>rs2055916</td>
<td>187433574</td>
<td>C</td>
<td>C</td>
<td>C</td>
<td>T</td>
<td>C</td>
<td>C</td>
</tr>
<tr>
<td>c.730C&gt;T (^c)</td>
<td>Exon 7</td>
<td>Q226X (^d)</td>
<td>187434513</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>c.755G&gt;C (^c)</td>
<td>Exon 7</td>
<td>R234T (^d)</td>
<td>187434538</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>c.801A&gt;G (^c)</td>
<td>Exon 8</td>
<td>rs5974</td>
<td>187438205</td>
<td>G</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>c.841C&gt;T (^c)</td>
<td>Exon 8</td>
<td>Q263X (^d)</td>
<td>187438245</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>c.1135+1G&gt;A (^c)</td>
<td>Intron J</td>
<td>−</td>
<td>187442240</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>c.1191T&gt;C (^c)</td>
<td>Exon 11</td>
<td>rs5970</td>
<td>187442295</td>
<td>C</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
<td>T</td>
</tr>
<tr>
<td>c.1481-34G&gt;T (^c)</td>
<td>Intron M</td>
<td>rs2289253</td>
<td>187444529</td>
<td>−</td>
<td>T</td>
<td>T</td>
<td>G</td>
<td>G</td>
<td>G</td>
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<tr>
<td>c.1556G&gt;A (^c)</td>
<td>Exon 15</td>
<td>W501X (^d)</td>
<td>187444638</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>c.1578C&gt;G (^c)</td>
<td>Exon 15</td>
<td>D508E (^d)</td>
<td>187444660</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>c.1812G&gt;T (^c)</td>
<td>Exon 15</td>
<td>rs5971</td>
<td>187446696</td>
<td>G</td>
<td>T</td>
<td>T</td>
<td>G</td>
<td>G</td>
<td>G</td>
</tr>
</tbody>
</table>

\(^a\)Markers used in Jewish or Italian haplotyping in previous studies are shaded in gray (27, 28).
\(^b\)Nucleotide number of the intronic polymorphisms have been assigned starting from the nearest splicing junction.
\(^c\)The approved Human Genome Variation Society (HGVS) nomenclature with ‘A’ of the translation initiation codon ATG numbered as +1 was used to describe nucleotide numbering for coding level.
\(^d\)Conventional numbering according to Asakai et al. (26) at the protein level omitting signal peptide counting start codon ATG as −18.

(CA)n, −431G>A, −361C>T, c.801A>G, c.1191 T>C and c.1812G>T which were previously used in Jewish and Italian haplotyping studies (14–15), −196T>G and c.1481-34G>T were also used to reconstruct haplotypes in Koreans in our study. Haplotype reconstruction and frequency estimation were performed using \textsc{phase} version 2.1 (http://www.stat.washington.edu/stephens/).

Statistical analysis

To show difference in haplotypes between the patient group and normal controls, chi-squared analysis was performed in chromosomes bearing Q263X or Q226X mutation and mutation-negative chromosomes using \textsc{spss} (Statistical Package for the Social Sciences, Chicago, IL) 14.0 for Windows. p-Value of <0.05 was considered as significant.

Results

Direct sequencing of all exons and their flanking introns from all patients revealed 13 distinct mutations (Table 1), including five novel missense mutations (Fig. 1a). In most patients (nine cases; 69%), mutations were identified during preoperative screening or routine physical check up. Others were found when examining for minor bleeding during surgical procedures or bleeding that is difficult to control (Table 1). Antigen levels were measured in patients with novel heterozygote mutations, and the levels corresponded to the FXI levels (Table 1). In the mutation analysis, the most frequently found mutations were Q263X (four cases; 31%) and Q226X (three cases; 23%) (Table 1). These two nonsense mutations were not found in 100 normal alleles. In this study, we identified five novel missense mutations: Q14R, R234T, H35Q, W383R, and D508E (Fig. 1a), which were not found in 100 normal alleles. All five were found at residues that are highly conserved across different species by using the \textsc{ClustalW} program (Fig. 1b). Furthermore, all five missense mutations were predicted to be pathogenic by two or more \textit{in silico} software program. Two missense mutations, R234L and R234I, were located at residue R234, and changing from arginine to lysine caused a disruption in normal mRNA splicing (7, 19). At residue W383, a nonsense mutation was found with W228X in a Chinese family with FXI deficiency which was caused by reduced secretion of F11 (20). In this study, we also observed previously reported mutations (11, 21–24) of C212R, V498M, c.1560dupG, W501X, G400V, and a splice site mutation c.1135+1G>A (Table 1). One novel sequence variation, c.150_1-149InsAT, presumably a polymorphism, was also found in case 6, but \textit{in silico} analysis using \textsc{Human Splicing Finder} version 2.4...
Fig. 1. Sequence analysis and evolutionary assessment of F11. (a) Sequence chromatogram of five novel mutations found in this study. The mutation sites are indicated by red arrows. (b) Alignments of factor XI amino acid sequences of different species using CLUSTALW. The sequences where the novel missense mutations occurred are conserved.

(http://www.umd.be/HSF/) revealed that the variation does not impose an effect on splicing.

To establish whether the Q226X and Q263X mutations are on different haplotypes, 10 variants found between intron A and exon 15 were used to reconstruct F11 haplotypes (Table 2). As a result, haplotypes bearing Q263X and Q226X in our patients were CTA10GCATTT and TTC9ACATGG, respectively (Table 3). The frequency of Q263X-bearing haplotype was significantly different between controls (2% of the normal alleles) and patients (31% of mutant alleles) (p = 0.001) (Table 3). The frequency of Q226X-bearing haplotype was 19% in controls and 27% in patients, but the difference was not significant (p = 0.596).

The haplotypes bearing Q263X (CA10GCATT) or Q226X (TC9ACATG) mutation differed from the haplotypes bearing the type II mutation (CA11GCGCG) in the Jewish and European populations (Table 3).

### Discussion

This study shows a high prevalence of the Q263X and Q226X mutations in the Korean population and that the mutation spectrum of F11 causing FXI deficiency in the Korean population is distinct from that of other populations including the Jewish or European populations.

The Q263X mutation has been reported repeatedly in Japanese and Chinese patients (9, 10, 12, 13). Structurally, the Q263X mutation codes for a stop codon after a sequence coding for the third apple domain of the FXI protein, causing truncation of the fourth apple domain which is important for dimerization and secretion of FXI and the serine protease domain (10).
The high frequency of the Q263X mutation in Korean patients with FXI deficiency and the presence of significant difference in the frequency of the mutation-bearing haplotype between the control and patient groups revealed a founder effect of the mutation.

The Q226X mutation has been reported in Japanese patients with FXI deficiency (11), but never been reported in Chinese. The Q226X mutation disrupts the third apple domain, which is crucial for ligand binding to platelets, heparin, and other factor molecules in addition to losing apple 4 and serine protease domains (11, 22, 25). Although the difference in the frequency of the haplotype bearing Q226X mutation between control and patient groups was not significant, the Q226X-bearing haplotype was relatively more frequent in the patient group, suggesting another possible founder mutation in the Asian population. Apparently, the haplotype surrounding the Q263X mutation was distinct from the haplotype of Q263X, showing that the two mutations originated from two different founders. It will be of great interest to study Japanese and Chinese patients with the same mutations to see if they share the mutation-bearing haplotypes and use this information in tracing the racial and historical relationship in different ethnicities.

The high frequency of the Q226X and Q263X mutations in our study suggests that testing for the presence of these two mutations should be the first genetic screening in Korean FXI-deficient patients. By analyzing exons 7 and 8 of F11 alone can detect up to 57.1% of the mutations, and by adding exon 13 to the analysis, the detection rate can be increased to 71.4%.

In conclusion, our study provides an opportunity to understand the mutation spectrum of F11 in the Korean population. The identification of a founder effect improves the genetic screening strategy to be followed and facilitates the clinical diagnosis of the disease.

Supporting Information

The following Supporting information is available for this article: Table S1. Nucleotide sequence of the primers used in PCR and direct sequencing of F11.

Additional Supporting information may be found in the online version of this article.

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