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Clinical manifestations and mutation spectrum of 57 subjects with congenital factor XI deficiency in China

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ABSTRACT

Congenital factor XI (FXI) deficiency is a rare bleeding disorder with unpredictable bleeding tendency. Few studies in a large cohort have been reported regarding associations between FXI activity (FXI:C) or genotypes and bleeding symptoms currently. This study characterized clinical manifestations and mutation spectrum of 57 subjects with FXI deficiency in China. Clinical data were collected and mutations were identified by direct sequencing and determined by mRNA analysis. The result revealed bleeding symptoms were only found in 12 patients (12/57, 21.1%) with severely reduced FXI:C, and prolonged bleeding post injury/surgery as well as easy bruising were the commonest bleeding manifestations presented in respective 5 cases (5/12, 41.7%). A total number of 37 mutations were identified including 19 missense mutations, 9 nonsense mutations, 6 splice site mutations and 3 small deletions. Among them, 4 missense mutations, 5 splice mutations, 3 small deletions and a nonsense mutation were newly detected. W228*, G400V, Q263* and c.1136-4delGTTG with a total frequency of 48.3% were the most four common mutations in Chinese patients. RT-PCR analysis was carried out and confirmed that both c.596-8T>A and c.1136-4delGTTG were pathogenic due to frameshift resulting in respective truncated proteins. Our findings suggested clinical manifestations had little to do with FXI:C or genotypes, which required further study. This study, the largest investigation of FXI deficiency in China revealed that the *F11* mutation spectrum of Chinese population was distinct from those of other populations earlier established.

1. Introduction

Factor XI (FXI) is a unique coagulation factor that recruits the intrinsic pathway by activating factor IX (FIX). The zymogen circulating in the plasma is stabilized by forming a complex with high-molecular-weight kininogen (HMWK) [1]. FXI is synthesized as a 160-KDa disulfide-linked dimer, with each identical monomer composed of four 90 or 91 amino acid repeats called apple domains in the N-terminus (AP1 to AP4 mainly offering the binding sites for other coagulation factors) and a C-terminal trypsin-like catalytic domain [2].

Congenital FXI deficiency is a rare bleeding disorder. It is prevalent in people of Jewish ancestry, for evidence shows that the carrier rate can reach 10% in Ashkenazi Jews [3–5], yet statistics of morbidity rate in other races is not clear currently. Its bleeding manifestations range from absence of symptoms to injury-related bleeding especially when it afflicts anatomic sites with increased fibrinolytic activity, such as nose, oral cavity, tonsils and urinary tract [3,6]. It is suggested that FXI plays a significant role in the down regulation of fibrinolysis by promoting activation of thrombin activatable fibrinolytic inhibitor (TAFI). In plasma an intact FXI (intrinsic) feedback loop can generate greater amounts of thrombin than generated by the tissue factor-FVIIa pathway, which is responsible for the activation of TAFI [7–9]. FXI activity (FXI:C) fails to predict clinical bleeding severity, and individuals with severe bleeding tendency do not necessarily show relatively low FXI:C level [10,11]. Currently, there is no detailed bleeding score system concerned in terms of FXI deficiency, yet a bleeding score system validated for the diagnosis of von willebrand disease type 1 is recommended for inherited bleeding disorders [12].

The FXI gene (*F11*) is located on the long arm of chromosome 4 (4q35), and is 50 kb in length with 15 exons and 14 introns. Up to 220 mutations have found till now according to the FXI mutation database [13]. It is published that the overwhelming majority of mutations are point mutations, among which missense and nonsense mutations take up nearly 70%, and can be easily verified by direct sequencing. Large deletions /duplications, on the other hand, are rarely seen in FXI deficiency compared to cases in Hemophilia, and limited technologies such as quantitative PCR or Gap PCR have been applied to detect this [14,15]. Currently, multiplex ligation-dependent probe amplification (MLPA)

Abbreviations: APTT, activated partial thromboplastin time; BAT, bleeding assessment tool; BDGP, Berkeley Drosophila Genome Project; CNVs, copy number variations; FXI, Factor XI; FXI:C, FXI activity; *F11*, FXI gene; MLPA, multiplex ligation-dependent probe amplification; TAFI, thrombin activatable fibrinolytic inhibitor; TG, thrombin generation.

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has been broadly used to detect copy number variations (CNVs) of target sequence or target gene and was confirmed to be reliable and accurate. However, as FXI deficiency is a rare bleeding disorder and MLPA kit evaluation is not available, we herein adopted the Accucopy technique that we have previously used in the detection of *F8* and *F9* to screen CNVs of *F11* gene [16,17].

Interestingly, considerable genetic heterogeneity occurs in various ethnic groups. Ashkenazi Jewish population commonly presents the E117* and F283L whereas C38R was found to be the predominant mutation in French Basques [5,15,18–22]. When it comes to the Asians, situation differs. Two mutations, Q226* and Q263* have been both reported in Japan, China and Korea though a specific population study of a large scale has not been carried out [23–27]. Particularly, a recent study indicates that these two mutations are most frequently found in Korea [28].

The aim of the study was to analyze the clinical manifestations and mutation spectrum of *F11* gene in China by studying 57 subjects with FXI deficiency.

2. Materials and methods

2.1. Subjects

This study was approved by the Ethics Committee of Ruijin Hospital. 57 successive subjects (17 males and 40 females, from 1 year to 81 years, median age 38 years) with FXI deficiency originated from different pedigrees and different parts of China were enrolled in the study. All the participants were Han Chinese. 54 patients had severe deficiency (FXI:C < 20 IU/dL) and 3 (patients 52, 53, 54) had partial deficiency. The median level of FXI:C was 2.5 IU/dL. A total of 26 heterozygous relatives were also identified, with no complaint of bleeding diatheses. A majority of propositi (47/57, 82.5%) were detected at a laboratory screening prior to surgeries or routine check-up while only 10 were referred for a bleeding tendency evaluation. Clinical manifestations in charge about individual's bleeding tendency was directly collected by physicians and recorded in a standard questionnaire using the consensus ISTH bleeding assessment tool (BAT) [12]. Another age and sex-matched group of healthy volunteers (n = 60; 43 females, median age 35 years) were also enrolled to compare with patients regarding bleeding scores by using Mann–Whitney U test.

2.2. Hemostatic assays

After obtaining informed consent from patients and their family members, citrated blood samples were collected, and platelet-poor plasma was obtained by centrifugation at 2500 *g* for 15 min, aliquoted and stored at −80 °C until use. FXI:C was performed by one-stage method on an ACL-TOP automatic coagulometer (Instrumentation Laboratory, MA, USA). Activated partial thromboplastin time (APTT) mixing test (a portion of patient's plasma mixed with equal volume of pooled plasma from healthy donors) was measured to screen out a possible secondary FXI deficiency or allo-antibody to FXI.

2.3. Genetic analysis of F11 gene

Genomic DNA was extracted from peripheral whole blood using the QIAamp DNA blood purification kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. Detection of genetic defects of the *F11* gene was carried out by directly sequencing on ABI 3700 sequencer (Biosystems, Foster City, CA, USA). CNVs of *F11* gene were tested by the AccuCopy technique in heterozygous individuals with low FXI:C levels (patients 56, 57) [16,17,29]. In total, two multiplex PCR panels were designed to amplify 14 target segments covering each exon except exon 9 as exons 8, 9 and 10 lay close to each other. An additional four reference fragments, named 2p, 10p, 16p and 20q, were also included in each PCR reaction (Table S1). Raw data were analyzed by GeneMapper 4.0 (ABI) and the height data for all specific peaks were exported into a Microsoft Excel file to calculate the copy number of each target segment in a similar way as described by Du et al. [29]. A normal dosage range for each target segment was recommended as 1.7 to 2.3. Novel mutations were screened in 50 normal volunteers, as well as filtered against the SNP database from the 1000 Genome Browser Tutorial [30] to rule out polymorphisms.

Numbering for DNA was based on the cDNA sequence (Genbank No. NM_000128.3) with nucleotide +1 corresponding to the A of the ATG translation initiation codon, as recommended by Human Genome Variation Society (HGVS) [31]. Mutations of amino acid changes were reported in accordance with current preferred nomenclature that started the first amino acid after signal peptides.

2.4. F11 mRNA analysis for two putative splice site mutations

Two putative splice site mutations c.1136-4delGTTG and c.596-8T>A were identified in pedigrees 14 and 32. Patient 14 harbored a homozygous mutation of c.1136-4delGTTG whereas patient 32 was a compound heterozygote inherited with a splice site mutation of c.596-8T>A as well as c.1136-4delGTTG. Therefore, c.596-8T>A was also studied in the father of patient 32, who only carried a single heterozygous mutation of c.596-8T>A.

Total RNA of fresh peripheral blood was isolated using TRIzol reagent (Life technologies, Carlsbad, CA, USA) after the removal of red blood cells, and the cDNA templates were synthesized using random primers, oligod$T_{(12-18)}$ and M-MLV Reverse Transcriptase according to manufacturer's instructions (Ambion, Life technologies, Carlsbad, CA, USA). The mRNA transcripts were amplified by nested PCR with the same pairs of PCR primers and PCR primers were designed regarding the respective splicing variations. Primers for the c.596-8T>A mutation were as follows: forward 5′- AGCAGCGATTTCTGGGTA-3′ (c.276-293, exon 4), reverse 5′-GTGTACTGGGCAATCCACTCT-3′ (c.782-802, exon 8). Primers for the c.1136-4delGTTG mutation were: forward 5′-GAAGGG AAGGGCAAGTGTTACT-3′ (c.1021-1042, exons 9-10), reverse 5′-TGGG TCGTTGAGAATCTGTGTAAT-3′ (c.1472-1495, exons 12-13). The PCR products were analyzed by electrophoresis on a 2% agarose gel and sequenced to identify the potential transcript patterns of aberrant splicing.

2.5. In silico analysis

The interactive biosoftware Alamut v2.7 (http://www.interactive-biosoftware.com), which consists of five different splice site prediction tools (SpliceSiteFinder-like, MaxEntScan, NNSplice, HumanSplicingFinder and GeneSplicer), as well as the Berkeley Drosophila Genome Project (BDGP) (http://fruitfly.org/seqtools/splice.html) were adopted to predict the effect of putative splice site mutations on normal splicing. In addition, to evaluate the functional effects of novel missense variations, multiple sequence alignment of FXI proteins from different species was performed from NCBI (http://www.ncbi.nim.nih.gov/protein) to assess patterns of sequence conservation. *In silico* analysis was also carried out using prediction software SIFT, Polyphen2 and PROVEAN to predict the potential effect of the mutations.

3. Results

3.1. Bleeding history

Though harboring rather low FXI:C, a majority of individuals demonstrated no bleeding symptoms and were not diagnosed until periodical check up or pre-surgical screening. No additional hemostatic defects were identified. Significant difference was identified between propositi (median 0, range 0–6) and healthy volunteers (median 0, range 0–2, p = 0.034) evaluated by ISTH-BAT score (Fig. 1). As shown in Table 1, bleeding histories were only mentioned in 12 patients (12/57, 21.1%) with epistaxis 3/57 (5.3%), easy bruising 5/57 (8.8%), bleeding from