CASE REPORT

Genetic analysis of a hereditary factor XII deficiency pedigree of a consanguineous marriage due to a homozygous F12 gene mutation: Gly341Arg

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ABSTRACT

Objective and Importance: To study the gene mutations of factor XII (FXII) in a Chinese family of consanguineous marriage with FXII deficiency and illuminate the possible molecular pathogenic mechanism. It will contribute to our comprehension of the pathogenesis of the disease.

Clinical presentation: The proband was a 26-year-old Chinese pregnant woman who was discovered, in a pregnancy test, with a prolonged activated partial thromboplastin time (APTT) at 61.6s (reference range, 29.0–43.0s).

Techniques: The FXII activity (FXII:C) and FXII antigen (FXII:Ag) were tested with clotting assay and ELISA, respectively. The FXII gene (F12) was amplified by PCR with direct sequencing. A ClustalX-2.1-win and four online bioinformatics software (PolyPhen-2, PROVEAN, SIFT, and Mutation Taster) were used to study the conservatism and harm of the mutation. The reference range of each test indicator in our laboratory was established with 150 healthy subjects.

Conclusion headings: The FXII:C and FXII:Ag of the proband were 12% and 10% (normal range, 72–113%), respectively. Gene sequencing detected a homozygous c.1078G > A point mutation in exon 10 resulting in a substitution of glycine 341 by arginine (Gly341Arg) in the proline-rich domain of FXII. Family study showed that her elder brother had the same phenotype and genotype with her. In addition, there were another six heterozygous members in her family. Both conservatism and bioinformatics results indicated the mutation probably had affected the function of the protein. We thought the Gly341Arg mutation was responsible for the decreased activity of FXII:C and FXII:Ag. And in vitro expression experiment is performed to elucidate the precise pathological mechanism of the mutation.

KEYWORDS
Gene mutation; factor XII deficiency; polymerase chain reaction; molecular mechanism

Introduction

Factor XII (FXII) is the initiator of intrinsic coagulation system. It is converted to activated FXII (FXIIa) by kallikrein digestion at Arg353-Val354 to initiate intrinsic coagulation pathway when contacted with a negatively charged surface. The FXII also plays a vital role in fibrinolysis regulation, complement activation, bradykinin production, and inflammation [1,2]. The mature FXII is a single-chain 80 kDa glycoprotein, composed of 596 amino acid residues, which is primarily synthesized in the liver then secreted into plasma at a level of approximately 30ug/ml [3]. The protein structure consists of a N-terminal heavy chain and a C-terminal light chain. The heavy chain contains two fibronectin-type (FT-I and FT-II) domains, two epidermal growth factor-like (EGF-I and EGF-II) domains, a kringle domain, and a proline-rich domain, while the light chain includes a catalytic domain [4]. The FXII gene (F12) is 12 kb and located on chromosome 5q33-qter composed of 14 exons and 13 introns [5].

Hereditary FXII deficiency is caused by F12 (GenBank: AF538691.1) mutation, usually detected, by chance, for a prolonged activated partial thromboplastin time (APTT) on health checkup or pre-operative screening, which always presents with a decreased FXII:C activity [6]. Generally speaking, the deficiency showed no distinct clinical symptoms, such as a bleeding tendency, but several clinical investigations were manifested, which was a risk factor for thrombosis [7–11]. Nevertheless, the association with thrombus, if there is any, is relatively weak [12,13]. According to the different depression of FXII antigen (FXII:Ag) level, this deficiency is divided into three types: a cross-reacting material (CRM)-negative group (FXII:Ag cannot be detected), a CRM-positive group (FXII:Ag is normal), and a CRM-reduced group (FXII:Ag reduced). Furthermore, the common gene polymorphism, 46T in the 5′-untranslated region, four bases upstream of the initiate ATG codon, is associated with a low translation efficiency and a decreased FXII level in the plasma compared to 46C [14].

We, herein, detected a patient with FXII deficiency from a consanguineous marriage family, then we studied the phenotype and genotype of the proband and her families, finally found a homozygous c.1078G > A point mutation in exon 10 of F12 resulting in a substitution of glycine 341 by arginine (Gly341Arg).
Patients and methods

Patients

The proband was a 26-year-old Chinese second pregnant woman who went to The First Affiliated Hospital of Wenzhou Medical University (China) for a pregnancy test. She had no history of bleeding or thrombosis, also there was not any abnormal symptom during her first pregnancy. Conventional coagulation screening revealed a prolonged APTT at 61.6s (reference range, 29.0–43.0s), which can be corrected with normal mixing plasma. Factor assays showed decreased FXII:C and FXII:Ag at 12% and 10% (reference range, 72–113%), respectively. Except the fibrinogen (FIB) and D-Dimer (D-D) were slightly high, other indexes, such as prothrombin time (PT), thrombin time (TT), factor VIII activity (FVIII:C), factor IX activity (FIX:C), and factor XI activity (FXI:C), were all within normal ranges (the data of FVIII:C, FIX:C, and FXI:C were not shown) (Table 1).

Family study showed that there was a consanguinity relationship between the proband’s parents, as her paternal grandmother and maternal grandmother were sister-german (Figure 1). A total of another 9 family members of 3 generations of her family participated in our research. Her elder brother had reduced FXII:C at 11% and FXII:Ag at 10%. Her father, mother, elder paternal aunt, elder maternal aunt, paternal grandmother, and maternal grandmother all had FXII:C and FXII:Ag decreased to approximately 30% and the younger paternal aunt and elder maternal uncle were with normal FXII:C and FXII:Ag. We also tested the APTT, PT, TT, FIB, and D-D of the 9 family members and found no abnormal results (Table 1).

Our study was approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University (China).

Methods

Coagulation index detection

All the APTT, PT, TT, FIB, D-D, and FXII:C were measured on a STA-R analyzer (DIAGNOSTICA STAGO, Asnieres-sur-Seine, France) using matched commercially available kits, of which the FXII:C was tested by a clotting assay. And the FXII:Ag was checked with enzyme linked immunosorbent assay (ELISA) kit (Changfeng, Wenzhou, China). All operations were carried out according to the manufacturers’ instructions. The reference ranges of these indexes in our laboratory were established with 150 healthy subjects.

DNA extraction and PCR amplification

To identify the molecular genetic background of FXII deficiency, genetic study for F12 was performed after obtaining the written informed consent from the proband, her family members, and 150 controls. Genomic DNA was extracted from peripheral whole blood using the QIAamp DNA Blood Mini kit (QIAGEN GmbH, Hilden, Germany) following the manufacturer’s instructions. All the 14 exons along with their intron–exon boundaries and 5′,3′-untranslated regions (reference sequence, GenBank: AF538691.1) were amplified by polymerase chain reaction (PCR) on a thermal cycler (Eppendorf, Hamburg, Germany) with primers as shown in Table 2.

The PCR system was 25ul containing GoTaq Green Master Mix (Promega Corporation, Madison, USA) (2x,

| Table 1. Phenotypes and genotypes of the inherited coagulation factor XII deficiency family. |
|-----------------|-------|-------|-------|-------|-------|-------|--------|
|                | PT (s)| APTT (s)| TT (s)| FIB (g/L)| D-D (mg/L)| FXII:C (%)| FXII:Ag (%)| Gly341Arg |
| IV2             | 12.6  | 61.6   | 14.9  | 5.19    | 0.61     | 12      | 10      | Homozygote |
| II1             | 12.4  | 37.9   | 16.1  | 3.10    | 0.20     | 34      | 32      | Heterozygote |
| II2             | 11.9  | 37.7   | 15.8  | 2.99    | 0.34     | 36      | 35      | Heterozygote |
| III1            | 12.8  | 39.0   | 15.2  | 3.41    | 0.23     | 34      | 37      | Heterozygote |
| III2            | 13.6  | 38.3   | 16.3  | 3.13    | 0.08     | 35      | 30      | Heterozygote |
| III3            | 13.3  | 35.8   | 17.9  | 3.45    | 0.09     | 99      | 97      | Wild type |
| III4            | 12.4  | 36.7   | 18.1  | 3.03    | 0.13     | 98      | 95      | Wild type |
| III5            | 13.3  | 35.4   | 15.8  | 3.05    | 0.36     | 35      | 38      | Heterozygote |
| III6            | 12.5  | 35.5   | 16.1  | 3.65    | 0.21     | 37      | 39      | Heterozygote |
| IV1             | 13.4  | 68.6   | 16.3  | 2.62    | 0.19     | 11      | 10      | Homozygote |

Normal range: 11.7–14.8 29.0–43.0 14.0–22.0 2.0–4.0 0–0.5 72–113 72–113
12.5ul), upstream primer (10uM, 1ul), downstream primer (10uM, 1ul), DNA template (100 ng, 2ul), and Nuclease-Free Water (8.5ul). The PCR reaction condition included these cycling steps: one cycle (95°C for 5 min), 30 cycles (95°C for 30s; annealing temperature for 30s; 72°C for 30s), and one cycle (72°C for 10 min).

DNA sequencing

The PCR products were detected by electrophoresis on a 1.5% agarose gel in Tris-borate-EDTA (TBE) buffer (pH 8.3) stained with GoldView II nucleic acid coloring agent (Lanji, Shanghai, China). After purification, qualified PCR products were directly sequenced on an ABI PRISM 3700 (Applied Biosystems, Foster City, USA). When mutational sites were detected, they were identified by reverse sequencing. The corresponding mutational exons of the family members were amplified and sequenced to confirm whether they carry the same mutation. We also sequenced the 46CT polymorphic site of 10 subjects.

 Conservation analysis

The multiple sequence alignment software ClustalX-2.1-win (Science Foundation Ireland, Dublin, Ireland) was used to analyze the conservative property of Gly341 in homo sapiens and its seven homologous species (Mus musculus, Rattus norvegicus, Pan troglodytes, Macaca mulatta, Canis lupus familiaris, Bos Taurus, and Xenopus tropicalis) (HomoloGene, http://www.ncbi.nlm.nih.gov/homologene).

Bioinformatics prediction

We adopted four online bioinformatics software to predict whether the amino acid substitution could affect protein function. They were ①Polymorphism Phenotyping v2 (PolyPhen-2, http://genetics.bwh.harvard.edu/oph2) (J. Craig Venter Institute, Tauranga, New Zealand), ②Sorting Intolerant From Tolerant (SIFT, http://sift.jcvi.org) (the Genome Institute of Singapore, Singapore, Singapore), ③Mutation Taster (http://www.mutationtaster.org) (Charité – Universitätsmedizin Berlin, Berlin, Germany). The reference protein ID of FXII was ‘P00748’ and the Ensembl transcript ID was ‘ENST00000253496’.

Results

DNA sequencing analysis indicated that the proband and her elder brother carried a homozygous c.1078G > A point mutation in exon 10 of F12 resulting in a substitution of glycine 341 by arginine (Gly341Arg) (Figure 2). Her father, mother, elder paternal aunt, elder maternal aunt, paternal grandmother, and maternal grandmother all took a heterozygous Gly341Arg. The younger paternal aunt and elder maternal uncle were the wild type of Gly341. Genetic results were in line with the coagulation index detection consequences (Table 1). Notably, the Gly341Arg missense mutation was absent in 150 healthy controls which suggested that it was not a common polymorphism. As for the 46CT polymorphism, except the father and the elder paternal aunt were heterozygotes of 46CT, all other eight family members were 46CC wild types.

Table 2. Polymerase chain reaction (PCR) primers for amplification of exons, exon–intron boundaries and 5′,3′-untranslated regions of factor XII gene.

| Exons | Primers | Product length | Annealing temperature |
|-------|---------|----------------|-----------------------|
| E1 + 2 | F: 5′-AGGGCAGCTTGACCAATC-3′ | 594bp | 60°C |
| | R: 5′-AGAGAAGCCAGGCCACTA-3′ | | |
| E3 + 4 | F: 5′-TGCTCTGAGGAGTTGATG-3′ | 437bp | 60°C |
| | R: 5′-ATGAGTGGGAGGAGGGA-3′ | | |
| E5 + 6 | F: 5′-CTCCTCCTCTCCACCATC-3′ | 434bp | 60°C |
| | R: 5′-CCCTTCCACAACATCTCG-3′ | | |
| E7 + 8 | F: 5′-CATGCCTGCCCTCTCCCACAG-3′ | 600bp | 58°C |
| | R: 5′-GTTAGAGGCGGCGGGAG-3′ | | |
| E9 + 10 | F: 5′-ATCCCCCTGTGTTCTT-3′ | 703bp | 58°C |
| | R: 5′-CCCCATCCCGTGTTCCAG-3′ | | |
| E11 + 12 | F: 5′-CTGGAAGGCGGAGGATG-3′ | 530bp | 56°C |
| | R: 5′-GGGGGCTTCCCTGCCAACAC-3′ | | |
| E13 + 14 | F: 5′-GCCGACGGTGTGCGAA-3′ | 638bp | 54°C |
| | R: 5′-GCCGACCCGATGAAGAAA-3′ | | |

Figure 2. The sequence diagram of Gly341Arg. A is the forward sequencing of homozygous c.1078G > A and B is the backward sequencing of homozygous c.1078C > T; C is the forward sequencing of heterozygous c.1078G > A and D is the backward sequencing of heterozygous c.1078 C > T. The position of mutational base is indicated with an arrow.
Table 3. The bioinformatics software’s prediction results of Gly341Arg.

| Software name | Prediction results | Score |
|---------------|--------------------|-------|
| PolyPhen-2    | Probable damaging  | 0.934 |
| PROVEAN       | Deleterious        | −6.214|
| SIFT          | Affect protein function | 0.01  |
| MutationTaster| Disease causing    | 0.976 |

The results of prediction are as in the table, and the meaning of scores is as follows:

1. PolyPhen-2: Scores are evaluated as 0.000 (most probably benign) to 1.000 (most probably damaging).
2. PROVEAN: The predefined threshold of score is −2.5, the score < −2.5 (deleterious), > −2.5 (neutral).
3. SIFT: Scores range from 0 to 1, the score < 0.05 (damaging), > 0.05 (tolerated).
4. MutationTaster: A probability close to 1 indicates a high security of prediction.

have an obvious influence on the decrease of FXII:C of this family. So, we preliminarily considered that the Gly341Arg mutation was responsible for the reduction of FXII:C. Besides, the reduced degree was lower in homozygotes compared to heterozygotes as what we had known before. Combined with Figure 1, we could see that the homozygous Gly341Arg alleles of the family were derived from her father and mother, and the mode of its inheritance is in accordance with that of autosomal recessive trait which is common in patients with hereditary FXII deficiency. Because the FXII:C and FXII:Ag of Gly341Arg were both reduced, there was still some antigen that could be detected, so this mutation belongs to the CRMRed group.

To further prove the adverse effect of Gly341Arg, we analyzed its conservatism and the possible effects on the protein. It turned out that Gly341 was a highly conservative site in the homologous species which was declared to be an important functional site of the FXII and played a vital role in the normal functions of the protein. And the four bioinformatics software all manifested the Gly341Arg, which was a deleterious mutation and could affect the FXII protein function, thus causing a corresponding disease. These results were strongly supported by our initial conclusion that the Gly341Arg mutation was responsible for the reduction of FXII:C.

Gly341Arg was located in the proline-rich domain of the heavy chain of FXII protein. This domain was a unique structure of FXII as opposed to other blood coagulation factors. It seems to play some role in the binding of FXII to negatively charged surfaces [15,16]. Thus, a speculation is that this mutation may influence the mechanisms of contact activation of FXII, then inappropriately facilitate FXII activation. There were another four mutations reported in this region: Thr309Lys, Thr309Arg [17], Ala324Pro [18], and Lys346Asn [19], of which the in vitro expression study of Lys346Asn indicated that the mutant protein was degraded intracellularly through endoplasmic reticulum-associated degradation as the protein quality control system resulted in insufficient secretion. As the Gly341 located adjacent to Lys346, we speculated that the decreasing mechanism of FXII:C and FXII:Ag caused by Gly341Arg was the same as Lys346Asn.

Because there was not a X-ray 3D structure file of the proline-rich domain of FXII, we were not able to do the spatial structure analysis of the mutant protein. But, we knew that Gly is a polar neutrally charged aminoacid, while Arg is polar positively charged. When Gly341 was replaced by Arg341, the charge modifications might affect the partial spatial conformation of the proline-rich domain. What is more, since the side chain of Arg341 was longer than that of Gly341, the extended side chain of Arg341 might form other intermolecular forces, such as hydrogen bond and steric effects with other amino acids, which are adjacent to

Figure 3. The conservative analysis diagrams of Gly341Arg. The targeted amino acid was indicated with an arrow, this line are all G(Gly) which indicated that the G(Gly) are conserved in the homologous species.

homologous species: Mus musculus, Rattus norvegicus, Pan troglodytes, Macaca mulatta, Canis lupus familiaris, Bos Taurus, and Xenopus tropicalis (Figure 3).

The forecasting results of these four bioinformatics software were ‘Probable damaging’, ‘Deleterious’, ‘Affect protein function’, and ‘Disease causing’ corresponding to ‘PolyPhen-2’, ‘PROVEAN’, ‘SIFT’, and ‘Mutation Taster’ with the scores ‘0.934’, ‘−6.214’, ‘0.01’, and ‘0.976’, respectively (Table 3).

Discussion

Family study of the proband indicated that she and her elder brother both had FXII:C and FXII:Ag approximately reduced to 10% and they took the homozygous Gly341Arg mutation. While the FXII:C and FXII:Ag of her father, mother, elder paternal aunt, elder maternal aunt, paternal grandmother, and maternal grandfather were all decreased to around 30% and they carried the heterozygous Gly341Arg mutation. The younger paternal aunt and elder maternal uncle with normal FXII:C and FXII:Ag were the wild type of Gly341. And that the depressed FXII:C could be corrected with normal mixing plasma. In addition, only her father and the elder paternal aunt carried a 46 T allele, but other eight members all took 46CC genotype which indicated that the 46CT polymorphism did not
the spatial structure with Arg341. The changed intermolecular forces would affect the normal space structure of the molecules, and translated into unstable protein which was susceptible to be degraded, and will finally cause the reduction in the activity and antigen of the FXII. Furthermore, Gly341 situated next to Cys340, when substituted with Arg341, might disturb the disulfide bond between Cys340 and Cys467, which might exert certain influence on the structure of protein.

Up to now, 38 mutations of FXII have been registered in The Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/gene.php?gene=F12), including missense/nonsense, splicing, regulatory, small deletions, small insertions, and gross deletions mutations. Our recent report on the Gly341Arg missense mutation enriched the database and we preliminarily analyzed its molecular pathogenic mechanism, which will contribute to the future researches about FXII mutations.

In summary, we reported a FXII missense mutation Gly341Arg which could cause FXII deficiency and we also probed its possible pathogenic mechanism. Finally, we drew the conclusion that this mutation was the probable reason for the decrease FXII:C and FXII:Ag. Now, we are performing an in vitro expression experiment to elucidate the precise pathological mechanism.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Ethics approval

Our study was approved by the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University (China).

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