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ORIGINAL ARTICLE

Factor XII deficiency: a clinical and molecular genetic study

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Abstract
Factor XII deficiency is a rare inherited disorder caused by clotting factor XII (FXII, F12) deficiency. It is often asymptomatic but can have both thrombotic and haemorrhagic symptoms. The aim of this study was to describe the spectrum of F12 gene mutations in a Russian population and learn more about the relationship between F12 variants and clinical phenotypes. We obtained and analysed genetic and clinical data from 33 apparently unrelated patients with FXII plasma levels below 60% and genetic data from 26 healthy controls with no history of FXII deficiency. Forty mutant alleles and six different deleterious substitutions were identified. Of these substitutions, three were major in the Russian population (c.-62C > T, c.-57G > C and c.1532-1G > A, total frequency 92.5%) and the three others (p.615 del C, c.1180_1181delCA, and CD218 TAT- > CAT p.Tyr218His) were rare and novel in the world population. Eight patients with mild FXII deficiency were found to be homozygous for a hypomorphic variant of functional polymorphism C46T and have no other deleterious substitutions in the F12 gene. Contrary to data in the literature, our study showed that mild haemorrhagic manifestations are common among patients with FXII deficiency.

Keywords FXII deficiency · F12 variants · Hemostasis

Introduction

Hageman's disease is a rare inherited disorder caused by clotting factor XII (Hageman factor, FXII) deficiency. Descriptions of clinical manifestations of FXII deficiency in the literature are extremely controversial. Even though FXII takes part in the intrinsic coagulation cascade, it is not usually associated with a clinical bleeding predisposition [1]. In contrast, since the initial publication of the paper by Ratnoff et al. [2], there have been many reports suggesting that FXII deficiency may actually predispose to thrombosis [3]. Nonetheless, analysis of the literature indicates that most patients with venous thrombosis and FXII deficiency have some additional risk factors of venous thrombosis [4]. The research into the clinical manifestations of FXII deficiency has become especially important in the context of experiments on mouse models. They indicate that in mice, a factor XII knock-out or inhibition protects from vascular occlusive events while having only a minimal impact on haemostasis [5]. These experiments have aroused wider interest in factor XII as a novel target for antithrombotic therapy. Nonetheless, the clinical manifestations may be different between mice and humans [6]. This observation makes clinical studies on patients with hereditary deficiencies of FXII relevant and important.

To date, 60 pathogenic variants in the *F12* gene have been described in the HGMD (Human Gene Mutation Database). Nonetheless, in addition to pathogenic variants, functional polymorphisms are of interest because in contrast to rare genetic variants, they determine FXII activity variation in the general population. For the *F12* gene, the only known functional polymorphism that causes a moderate FXII deficiency in the homozygous state is c.-4 C > T. The frequency of the T allele (hypomorphic variant) is significantly higher in the Asian population than in the European population, thereby explaining significantly lower levels of plasma coagulant activity of the Hageman factor in the healthy Asian population compared to Europeans [7].

The aim of this study was to describe the *F12* gene mutation spectrum in a Russian population and learn more about

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the relations between genetic variants of the *F12* gene and clinical phenotypes.

Materials and methods

We obtained and analysed genetic and clinical data from 33 apparently unrelated patients with FXII plasma levels below 60%. Patients participating in the study were referred to the National Medical Research Center for Hematology (Moscow, Russia) in three different ways: patients have sought medical advice regarding blood coagulation issues (either haemorrhagic or thrombotic symptoms) ($n = 11$), patients have been discovered by accident through a routine pre-surgical coagulation test ($n = 19$), and asymptomatic patients with a family history of FXII deficiency ($n = 3$). To estimate T allele frequency of functional polymorphism c.-4 C > T in a healthy population, we also used genetic data from 26 conditionally healthy controls without a history of FXII deficiency who contacted the Center for other health problems (e.g. acute hepatic porphyria, acute lymphoblastic leukaemia or haemophilia).

All the patients provided written informed consent. The present work was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and with guidelines of a local ethics committee for experiments involving humans.

Total DNA was isolated from the whole-blood samples by standard proteinase K–SDS digestion and phenol–chloroform extraction.

We analysed all functionally important regions of the factor XII gene, i.e. the promoter region, all exons and exon–intron junctions, by Sanger sequencing. For amplification of target fragments, we used primers designed in our laboratory (Table 1); all primer pairs have an annealing temperature of 62 °C. PCR was carried out using the MasterMix (ThermoScientific, Waltham, MA, USA), following the manufacturer's protocol, in a 25 µl reaction containing 50–100 ng of the DNA template. PCR products were analysed by polyacrylamide gel electrophoresis and then purified using the Wizard PCR Preps DNA purification system (Promega, Madison, WI, USA). Sequencing was performed by means of ABI PRISM® BigDye™ Terminator v.3.1 on an Applied Biosystems 3730 DNA Analyzer (Applied Biosystems, Waltham, MA, USA). The sequencing data were analysed in SeqScape (Applied Biosystems) and aligned manually; as a reference, we used the nucleotide sequence of the human *F12* gene, GenBank accession No. NG_007568. For amino acid nomenclature, we used cDNA sequence NM_000505.3.

We analysed a single patient using the TruSight One sequencing panel (Illumina) following the manufacturer's protocol.

The pathogenicity of novel substitutions was interpreted using the standards, guidelines and nomenclature of HGVS and the following software packages: SIFT v.6.2.1, PROVEAN v.1.1.5, PolyPhen-2 v.2.2.2 and MutationTaster.

For activated partial thromboplastin time (APTT) and FXII activity measurements, we employed a CA-660 automated coagulation analyser (Sysmex, Milton Keynes, UK), the Pathromtin® SL aPTT reagent and coagulation-factor-deficient plasma FXII (Siemens Healthcare GmbH, Erlangen, Germany).

For the measurement of Hageman factor (FXIIa)-dependent fibrinolysis, we used reagents from RENAM (Moscow, Russia). The protocol included the following steps. A mixture of 8 ml of distilled H_2O, 0.2 ml of 1% acetic acid, 0.5 ml of blood plasma and 0.5% of kaolin was prepared. The mixture was carefully stirred and incubated at 37 °C for 30 min and then centrifuged for 6 min at 1500 rpm. The precipitate was dissolved in 0.5 ml of 50 mM Tris–HCl (pH 7.4) containing 130 mM NaCl. Clot formation was induced by the addition of an equal volume of 0.025 M calcium chloride.

Table 1 PCR primer sequences. In primer names, we use index F for forward primers and R for reverse

Name	Primer sequences (5′–3′)	Position (in NCBI NG_007568)	Location	PCR product size (bp)
F12F1	gct ttc caca aa cag cct gt	4920–4939	Promoter region + Exons 1, 2	680
F12R1	gac tgc aca cac tgc acc at	5599–5580		
F12F2	cct ttt cct gac cag acc ct	8433–8452	Exons 3, 4	566
F12R2	ctt ccc aga act ctc cct ct	8998–8979		
F12F3	ccc agc tgt gtg act cag ct	9090–9109	Exons 5–7	754
F12R3	cac tct ccc tcc tcc ttc ct	9843–9824		
F12F4	cag gaa gga gga gg gaga gt	9823–9842	Exons 8–10	978
F12R4	ggt tcg ggt gca gcg tgg aa	10,800–10,781		
F12F5	acc ctt ctt tcc acg ccc ct	10,824–10,843	Exons 11, 12	638
F12R5	cac att ctc aca acc cat ca	11,461–11,442		
F12F6	cct gga gca gct ttg tcc at	11,802–11,821	Exons 13, 14	638
F12R6	tc tca gca ttt tca aag cac	12,439–12,419		

After clot formation, the time of complete clot lysis was determined [8].

Local reference ranges for the coagulation variables were as follows: APTT, 29–38 s; FXII, 70–150%; and XIIa-dependent fibrinolysis, 5–12 min.

Statistical significance of differences in polymorphism frequencies was assessed by the χ^2 test in StatSoft 10.

Results and discussion

Six different pathogenic variants were identified in 25 individuals (16 patients with two pathogenic variants, 9 heterozygous patients, 8 patients without any deleterious substitutions). Three of them are known and widespread in the world population. Three variants are novel and have not been previously documented in the HGMD.

Microdeletion c.615delC in exon 7 [$N_{chrom} = 1$ (2.4%)] was found for the first time. This mutation produces a frameshift followed by a stop codon after 44 alternate codons downstream: p.(Gly206GlufsTer45). This frameshift mutation leads to the loss of the kringle domain and the entirety of the light chain of FXII.

The c.1180_1181delCA microdeletion in exon 10 [$N_{chrom} = 1$ (2.4%)] has not been previously reported either. This frameshift mutation results in a stop codon after 38 alternate codons: p.(His394GlnfsTer39).

We also detected a novel missense substitution, c.652T > C p.(Tyr218His) [$N_{chrom} = 1$ (2.4%)], in exon 9 (in the kringle domain). This variant was predicted to be deleterious by three out of four in silico analyses (Table 2) and can be interpreted as likely pathogenic based on ACMG criteria (PM2, PP3) [9].

Major mutation c.1532-1G > A [$N_{chrom} = 19$ (46.3%)] has been previously found in European populations [10]. The mutation is located at the 3′ splice acceptor site of exon 14. It creates a new acceptor site one nucleotide downstream of the natural one, which as a consequence shifts the reading frame in exon 14 one nucleotide downstream. Accordingly, the protein lacks the amino acid residues encoded by exon 14, including the functionally important translational stop codon, resulting in a detectable transcript but an unstable protein [11].

Substitutions c.-62C > T [$N_{chrom} = 7$ (17%)] and c.-57G > C [$N_{chrom} = 12$ (29.3%)] have also been described in the European population. We found them in six and eight patients, respectively. Both mutations are located in the promoter region of *F12* at a putative binding site for HNF4α. Both cause significant underexpression of *F12* [12].

For comparison of the patients' phenotypes, we used only one coagulation parameter: FXII activity. APTT and XIIa-dependent fibrinolysis are strongly related to the FXII activity level (Fig. 1), indicating that these metrics are not independent.

Table 2 Pathogenicity predictions for novel variant c.652T > C p.(Tyr218His)

Software	Score	Prediction	Cut-off
MutationTaster	0.983	Polymorphism	
PolyPhen-2	1	Probably damaging	
PROVEAN	− 3.67	Deleterious	− 2.5
SIFT	0.005	Damaging	0.05

F12 activity vs. the genotype

The results of the F12 gene sequencing allowed us to subdivide our patients into three groups: patients with two mutations in this gene (homozygous or compound heterozygous

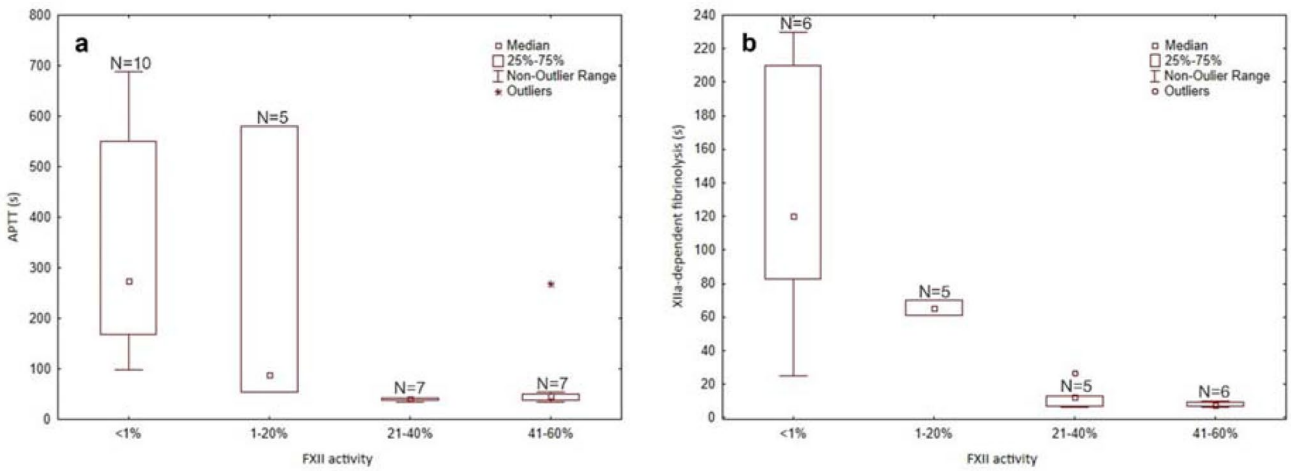

Fig. 1 APTT (**a**) and XIIa-dependent fibrinolysis (**b**) in groups with different FXII activity

mutations, $n=16$), patients heterozygous for F12 mutations ($n=9$) and patients without any deleterious substitutions in this gene ($n=8$). It has been demonstrated that homozygous and compound heterozygous patients exhibit almost no factor XII activity, whereas heterozygotes have intermediate values (Table 3). Our data regarding FXII activity are in agreement with the expected tendency.

The patients without any deleterious substitutions show an average FXII activity of 44.5%. All of them, except one, were homozygous for the T allele at the c.-4 position. This may be the reason for a mild FXII deficiency in this group of patients. This hypothesis is supported by the finding that in the group of patients with reduced FXII activity, the frequency of allele C at c.-4 is 0.197 (Nchrom = 66), which is significantly lower than that in the control group: 0.615 (Nchrom = 52; $\chi^2=21.583$, $p<0.000$, df = 1). In our study, the frequency of the C allele (0.615) in the control group was intermediate between European and in Asian populations (0.75 and 0.39, respectively, according to gnomAD).

Thus, the reduction in FXII activity is caused by two kinds of factors: the presence of pathogenic variants in the F12 gene and the homozygosity for a hypomorphic variant of functional polymorphism c.-4C > T.

In this work, we identified only one patient with FXII deficiency (56%) and no abnormalities in the F12 gene. For this patient, we performed an additional analysis and tested 5000 genes by clinical exome next-generation sequencing. We did not find any genetic variations that could cause the FXII deficiency. These data indicate that the regulation of the FXII activity is a complex process that can include non-obvious mechanisms, both hereditary and epigenetic.

Clinical picture vs. mutations

We performed analysis of relationships between genetic variants of F12 gene and clinical phenotypes for 30 patients. We excluded three patients from the analysis due to them having accompanying FXII deficiency with either a mutation in the F8 gene, or with a mutation in the FGA.

All clinical manifestations of coagulation impairment observed in our patients were classified into two categories. The first group included haemorrhage complications: bleeding gums, epistaxis, easy bruising, single abnormal bleeding, bleeding in a postoperative period or menorrhagia. The second group included thrombotic events: thrombophlebitis. Detailed information for each patient are described in Supplementary Table 1.

In a large proportion of patients ($n=20$), mild or moderate haemorrhagic manifestations of FXII deficiency were observed during their lifetime (Table 4). Six of them have sought medical advice regarding haemorrhagic manifestations. In one case, haemorrhagic symptoms were partly related to non-steroidal anti-inflammatory drugs (NSAIDs), whereas in the other six patients who occasionally took an NSAID, either there was no bleeding at all ($n=4$) or the bleeding episodes were not connected with the use of NSAIDs ($n=2$). Two patients had cases of thrombosis, one of which was associated with an additional provoking factor (thrombophlebitis after installation of a catheter). It is also noteworthy that bleeding and thrombosis symptoms in the patients that have sought medical advice regarding blood coagulation issues are only a little more clinically prominent than the symptoms in the patients who have been discovered by accident through a routine pre-surgical coagulation test (Supplementary Table 1).

Our sample does not allow us to unambiguously investigate relations between specific pathogenic variants and clinical manifestations, because genetic variants are mostly present in combinations with each other. Only a few trends can be discerned.

Patients homozygous for the T allele at the c.-4 position who have no mutations in the F12 gene demonstrate a wide variety of clinical manifestations and a decrease in FXII activity to 26–58% (Table 4).

All three common mutations (c.-62C > T, c.-57G > C and c.1532-1G > A) in the homozygous state result in very low or no FXII activity. When these mutations are heterozygous and are in a compound state with each other or with different mutations in the F12 gene, FXII activity is low too. The only exception is one patient, who is a compound heterozygote of mutations c.-62C > T and c.615delC and has 24% FXII activity. It is possible that in this case, both substitutions are located in the same allele; however, no pedigree data are available for this patient, and therefore, no segregation analysis can be performed. In a heterozygous state, mutations c.-62C > T, c.-57G > C and c.1532-1G > A induce mild FXII deficiency, which can become moderate when they are combined with the T allele at c.-4. Only in one heterozygous patient (genotype c.1532-1G/A, c.-4T/T) did we see severe FXII deficiency (0.7%), which could be caused by an additional mutation, which could not be identified by the methods utilised in this work (for example, an extended deletion or a deep intronic mutation that disrupts normal splicing).

Table 3 Median FXII activity in patients from different genetic cohorts

Cohort	No. of patients	Median (range) of FXII, %
Patients with two mutations	16	0.5 (0–24)
Heterozygous patients	9	31.5 (1.5–72.0)
No substitutions[a]	8	42.3 (26.7–58.0)

[a]Patients carrying polymorphism c.-4C > T (see below) were included in this category if they did not have any other substitutions

Table 4 Genetic characterisation of different clinical manifestations in the studied patients

Variants of F12 gene	Number of patients	Mean FXII activity	Range of FXII activity	Asymptomatic patients	Patients with haemorrhagic symptoms	Patients with both haemorrhagic and thrombotic symptoms
c.-4 T/T	7	42.9	(26.7–58.0)	1	3	2
c.-62 T/T c.-4 C/C	1	4.5	–			
c.-62 C/T c.-4 C/C	1	72	–		1	
c.-62 C/T c.-4 C/T	1	42.9	–	1		
c.-62 C/T c.-4 C/T c.1532-1G/A	1	4.9	–	1		
c.-62 C/T c.-4 C/T c.1180_1181delCA hetero	1	8.4	–	1		
c.-62 C/T c.-4 C/C c.615 del C hetero	1	24	–	1		
c.-57 C/C c.-4 T/T	3	0.2	(0–0.5)		3	
c.-57 C/C c.-4 C/T	1	0.1	–	1		
c.-57 G/C c.-4T/T	1	18	–		1	
c.-57 G/C c.-4 C/T c.652T > C hetero	1	0.5	–		1	
c.-57 G/C c.-4T/T c.1532-1G/A	2	0	–		2	
c.1532-1A/A c.-4T/T	5	0.7	(0.1–0.5)	2	3	
c.1532-1G/A c.-4T/T	6	26.4	(1.5–45.0)	1	4	
c.-4 C/C	1	56	–	1		
Total	33			10	18	2

In addition, we note that out of 22 women in the study population, 5 had a miscarriage. Association between factor 12 deficiency and miscarriage have been noted in literature [13].

We showed an influence of pathogenic and hypomorphic variants in the F12 gene and of their combinations on the level of FXII activity. Nonetheless, we failed to identify any relation between clinical manifestations and pathogenic variants. Nevertheless, it is noteworthy that in contrast to literature data, in our study, mild haemorrhagic manifestations are common among patients with FXII deficiency.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12185-023-03535-9.

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Data availability All data associated with this study are available in the main text, its supplementary materials and through the corresponding author upon request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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