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Exploring the global landscape of genetic variation in coagulation factor XI deficiency

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Key Points

- Exome-data analysis revealed that FXI deficiency is from 2 to 20 times more frequent than expected in most populations.
- Exome-data analysis evidenced novel recurrent and ethnic-specific mutations other than the well-known type II and type III defects.

Factor XI (FXI) deficiency is an autosomal bleeding disorder, usually posttrauma or postsurgery, characterized by reduced levels of coagulation FXI in plasma. The disease is highly prevalent in Ashkenazi Jews (heterozygote frequency, ~9%), whereas it is considered a rare condition in most populations (prevalence of the severe deficiency, 1 in 10^6 in the white population). So far, >190 causative mutations have been identified throughout the *F11* gene. To have a global landscape of genetic variation of *F11*, we explored publicly available exome-based data obtained from >60 000 individuals belonging to different ethnicities (Exome Aggregation Consortium resource). This analysis revealed profound differences in heterozygote frequencies among populations (allele frequencies: African = 0.0016; East Asian = 0.0045; European = 0.0036; Finnish = 0.00030; Latino = 0.0021; South Asian = 0.0015), and a prevalence significantly higher than that reported so far (eg, the calculated prevalence of the severe deficiency in Europeans would be: 12.9 in 10^6). In addition, this analysis allowed us to evidence recurrent and ethnic-specific mutations: p.Phe223Leu in Africans (23.5% of all mutated alleles), p.Gln263X and p.Leu424CysfsX in East Asians (28.2% and 20.5%, respectively), and p.Ala412Thr in Latinos (25%). (*Blood*. 2017;130(4):e1-e6)

Introduction

Coagulation factor XI (FXI) is a 160-kDa glycoprotein mainly synthesized in hepatocytes and secreted into the circulation as a zymogen.[1] In the coagulation cascade, activated FXI (FXIa) plays a fundamental role in the activation of factor IX, a key step for thrombin generation at the site of vessel injury.[1]

Hereditary FXI deficiency (Mendelian Inheritance in Man, MIM*264900) is an autosomal hemorrhagic disorder characterized by mildly to severely reduced levels of coagulation FXI in plasma. The deficiency can be classified as quantitative or qualitative, based on concordance/discordance of FXI antigen and activity levels.[2] In severe FXI-deficient patients (ie, those showing FXI levels <20 IU/dL), the disease is usually associated with mild-to-moderate bleedings, principally after trauma or surgery. The bleeding history of patients with partial FXI deficiency is often unpredictable, making them not easily distinguishable from the severe ones.[3] Genotype-phenotype correlation in these patients is also jeopardized by the possible coexistence of other hemostatic defects.[4]

Hereditary FXI deficiency is mostly associated with genetic defects in the FXI gene (*F11*), which is composed of 15 exons spanning ~24 kb on 4q35.2. According to the FXI Deficiency Mutation Database (http://www.factorxi.org/),[5] >190 disease-causing mutations have been reported, with a great preponderance of missense mutations (66%). A high level of allelic heterogeneity is found in most populations, where prevalence of 1 in 10^6 has been reported.[2] On the other hand, important founder effects have been reported in specific populations, associated with higher prevalence. In particular, prevalent ancestral mutations were found in Basques from France (p.Cys38Arg), in French patients from Nantes (p.Gln88X), in English patients (p.Cys128X), and, above all, in Ashkenazi Jews (the so-called type II p.Glu117X and type III p.Phe283Leu mutations).[6-9] Indeed, 1 in 450 Ashkenazi Jews are expected to suffer from type II homozygous, type III homozygous, or type II-III compound heterozygous FXI deficiency.[9]

In this work, we attempted to define the global landscape of *F11* genetic variation and to determine population-specific carrier rates in FXI deficiency by exploring exome data available through the Exome Aggregation Consortium (ExAC) resource.

Methods

Calculations of global exome-based prevalence of FXI deficiency

For assessing prevalence of FXI deficiency in different ethnic groups, we extracted data from the ExAC database, which contains exome data from 60 706

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Table 1. Estimated global exome-based prevalence of FXI deficiency by ethnicity

Population	Total no. of alleles	Total no. of variants	Collective frequency of variants	Heterozygote frequency	Homozygote/compound heterozygote frequency in 1000 individuals	Calculated prevalence of FXI deficiency in 10^6 individuals
All	121 412	420	0.0035	0.0069	0.0120	12.0
East Asians	8 654	47	0.0054	0.0108	0.0295	29.5
Others	908	4	0.0044	0.0088	0.0194	19.4
Europeans*	66 740	271	0.0041	0.0081	0.0165	16.5
Latinos	11 578	36	0.0031	0.0062	0.0097	9.7
Africans	10 406	25	0.0024	0.0048	0.0058	5.8
South Asians	16 512	32	0.0019	0.0039	0.0038	3.8
Finns	6 614	5	0.0008	0.0015	0.0006	0.6

The carrier rates of FXI deficiency were estimated using the ExAC data set, including all null mutations plus missense variants predicted to be deleterious by 7 of 7 prediction software (see "Methods").

*Non-Finnish Europeans.

unrelated individuals sequenced as part of several disease-specific studies (not including FXI deficiency), as well as population-based genetic studies.[10]

Among the reported variants, we considered only: (1) disruptive mutations (nonsense, frame-shift, and splice-site variants, the latter including only those affecting the first 2 or last 2 intronic nucleotides) and (2) nonsynonymous variants annotated as deleterious by 7 of 7 prediction programs. These software, all comprised in the dbNSFP database, were: SIFT,[11] PolyPhen2 (2 different algorithms),[12] MutationTaster,[13] MutationAssessor,[14] the Likelihood Ratio Test (LRT),[15] and the Functional Analysis Through Hidden Markov Model (FATHMM).[16]

We performed prevalence calculations using 2 different approaches. In one case, we considered all variants belonging to groups 1 and 2. In the other case, we restricted the mutation list to group 1 and to those variants of group 2 already described in FXI deficiency, by searching them in publicly available databases: the Factor XI mutation database, the Leiden Open-source Variation database (LOVD; http://www.lovd.nl/3.0/home),[17] the Expert Protein Analysis system (ExPASy; https://www.expasy.org/),[18] the ClinVar resource (http://www.ncbi.nlm.nih.gov/clinvar/),[19] and the public release of the Human Gene Mutation Database (HGMD; http://www.hgmd.cf.ac.uk/ac/index.php).[20]

Results

Global carrier rates of FXI deficiency using population-based exome-sequencing data

The ExAC database offers information on sequence variation in 60 706 unrelated individuals collected through the contribution of 16 exome-based projects.[10] High-quality variants were collected in subjects of diverse ethnicities, including Africans, non-Finnish Europeans, Finnish Europeans, admixed Americans (Latinos), South Asians, East Asians, and other ethnicities. Data on phenotypes for these individuals are not available, but it is known that those suffering from severe pediatric diseases were not included.

Among the reported variants, we retrieved all of the null mutations as well as the nonsynonymous variants annotated as damaging by 7 of 7 prediction programs. We found a total of 420 variants in 121 412 different alleles (Table 1); the number of variants decreased to 351 considering only those mutations that have already been reported as associated with FXI deficiency in publicly available repositories (Table 2; in Table 3, we listed the mutations predicted as dangerous by 7 of 7 prediction programs, but not annotated in the literature). All variants were present in heterozygous individuals, with the exception of 1 subject (European, non-Finnish) who was homozygous for the type II mutation.

In general, our analysis revealed prevalence data for severe FXI deficiency significantly higher than those reported so far. For instance, non-Finnish Europeans show a rate of 12.9 in 10^6, East Asians display the exceptional rate of 20.3 in 10^6, whereas for Latinos we observed a rate of 4.6 in 10^6. Africans and South Asians show lower and similar rates (around 2 in 10^6), whereas FXI deficiency seems to be quite rare among Finns (0.1 in 10^6) (Table 2).

Concerning specifically the type II and type III mutations, these were confirmed to be present almost exclusively in Europeans, with the only exception of South Asians, in which the type II mutation was quite frequent (12.5% of all mutated alleles) (Table 4).

Finally, this analysis allowed us to highlight at least 4 recurrent and ethnic-specific mutations: the missense variant p.Phe223Leu in Africans (23.5% of all mutated alleles), the nonsense p.Gln263X and the frameshift p.Leu424CysfsX mutations in East Asians (28.2% and 20.5% of all mutated alleles, respectively), and the missense variant p.Ala412Thr in Latinos (25% of all mutated alleles) (Table 4).

Discussion

The genetic bases of quantitative FXI deficiency are almost invariably constituted by mutations within the *F11* gene. The best source of publicly available information concerning FXI deficiency–causing mutations is the FXI Deficiency Mutation Database.[5] Since 1999, 191 mutations have been reported, highlighting the high allelic heterogeneity of the disease. The absence of important symptoms, often characterizing FXI-deficient patients, could be responsible for an underestimation of the actual prevalence of the disease. In this respect, genetic-epidemiologic research could be of help to improve these estimations. Of course, this kind of approach has remained largely unexplored in the case of rare diseases, mainly due to the absence of a significant amount of genetic data. This was particularly true for countries with limited resources, considering that most epidemiologic studies have been performed on individuals from North America and Europe.[21,22] Today, the progress in next-generation sequencing technologies is revolutionizing the field of genetic epidemiology, with huge amounts of exome data from large international consortia becoming increasingly available. Hence, we decided to explore the global landscape of genetic variation in FXI deficiency by taking advantage of the extraordinary resource represented by the ExAC database, which offers information on sequence variation in >60 000 individuals, including those coming from "developing" countries.

In ExAC, all of the 14 protein-coding exons of the *F11* gene were sequenced at sufficient depth (on average 69× coverage, ~10% of individuals with coverage >100×), with data available for a total of 121 412 alleles. From this data set, we retrieved 420 potentially

Table 2. Estimated global exome-based prevalence of FXI deficiency by ethnicity: mutations included only if present in FXI deficiency patients in public databases

Population	Total no. of alleles	Total no. of carriers	Collective frequency of variants	Heterozygote frequency	Homozygote/compound heterozygote frequency in 1000 individuals	Calculated prevalence of FXI deficiency in 10^6 individuals
All	121 412	351	0.0029	0.0058	0.0084	8.4
East Asians	8 654	39	0.0045	0.0090	0.0203	20.3
Others	908	4	0.0044	0.0088	0.0194	19.4
Europeans*	66 740	240	0.0036	0.0072	0.0129	12.9
Latinos	11 578	24	0.0021	0.0041	0.0040	4.3
Africans	10 406	17	0.0016	0.0033	0.0027	2.7
South Asians	16 512	25	0.0015	0.0030	0.0023	2.3
Finns	6 614	2	0.0003	0.0006	0.0001	0.1

These "more conservative" carrier rates of FXI deficiency were estimated using the ExAC data set, including all null mutations plus missense variants previously reported as associated with FXI deficiency in public databases (see "Methods").

*Non-Finnish Europeans.

deleterious alleles, corresponding to 96 different null mutations or missense variants predicted to be damaging by 7 of 7 algorithms. Their distribution was 25% nonsense, 60.4% missense, 6.3% splicing, and 8.8% frameshift mutations. This distribution was significantly different ($\chi^2 = 11.4$; 2-tailed $P = .0098$) from that reported in the FXI Deficiency Mutation Database (13.7% nonsense, 66.3% missense, 9.5% splicing, 10.5% frameshift mutations; the "undefined" mutation and the variant located in the promoter region were excluded from this calculation), essentially for an inflation of nonsense mutations, which, however, have a clear impact on the protein. For the sake of comparison, we also compared the mutation type distribution between the ExAC variants predicted to be damaging by all algorithms and those listed in the FXI Deficiency Mutation Database that were "predicted" by the same algorithms to be deleterious. Though annotated in the

Table 3. Mutations predicted as dangerous by 7 of 7 prediction programs

RefSeq	Genomic position*	cDNA level†	Native protein	Mature protein
	Chr4:187 192 766	c.59G>C	p.C20S	p.C2S
	Chr4:187 192 805	c.98G>A	p.G33E	p.G15E
	Chr4:187 192 867	c.160C>T	p.P54S	p.P36S
	Chr4:187 194 248	c.242G>T	p.S81I	p.S63I
	Chr4:187 194 287	c.281C>T	p.A94V	p.A76V
	Chr4:187 194 313	c.307T>C	p.C103R	p.C85R
	Chr4:187 195 366	c.422C>A	p.T141K	p.T123K
	Chr4:187 195 380	c.436T>C	p.C146R	p.C128R
	Chr4:187 195 398	c.454G>A	p.A152T	p.A134T
rs142929551	Chr4:187 195 410	c.466T>C	p.F156L	p.F138L
	Chr4:187 197 005	c.550G>A	p.V184M	p.V166M
	Chr4:187 197 481	c.692C>G	p.T231S	p.T213S
	Chr4:187 197 510	c.721T>G	p.F241V	p.F223V
	Chr4:187 201 167	c.757A>T	p.N253Y	p.N235Y
	Chr4:187 201 428	c.917T>A	p.L306Q	p.L288Q
	Chr4:187 201 500	c.989T>G	p.F330C	p.F312C
	Chr4:187 201 650	c.1051T>C	p.S351P	p.S333P
	Chr4:187 205 249	c.1139G>A	p.C380Y	p.C362Y
rs149689934	Chr4:187 205 316	c.1206G>T	p.Q402H	p.Q384H
rs121965071	Chr4:187 205 363	c.1253G>A	p.G418D	p.G400D
	Chr4:187 205 369	c.1259T>C	p.I420T	p.I402T
	Chr4:187 206 931	c.1444G>A	p.A482T	p.A464T
	Chr4:187 206 932	c.1445C>T	p.A482V	p.A464V
	Chr4:187 207 630	c.1542C>G	p.C514W	p.C496W
	Chr4:187 207 641	c.1553G>A	p.G518E	p.G500E
rs139695003	Chr4:187 208 874	c.1613C>A	p.P538H	p.P520H
	Chr4:187 208 906	c.1645T>C	p.Y549H	p.Y531H
	Chr4:187 208 907	c.1646A>G	p.Y549C	p.Y531C
	Chr4:187 208 973	c.1712G>C	p.C571S	p.C553S
	Chr4:187 209 610	c.1720G>T	p.D574Y	p.D556Y
rs149873248	Chr4:187 209 617	c.1727G>A	p.G576E	p.G558E
	Chr4:187 209 673	c.1783T>A	p.W595R	p.W577R
	Chr4:187 209 677	c.1787G>A	p.G596D	p.G578D

These 33 missense defects, which appear in the ExAC data set in at least 1 individual, were predicted to be deleterious by 7 of 7 prediction software (see "Methods"), and are not annotated in any FXI-related public repository.

cDNA, complementary DNA.

*Numbering according to University of California Santa Cruz (UCSC) Genome Browser, human, February 2009 (GRCh37/hg19) assembly.

†Numbering starting from ATG according to NM_000128.

Table 4. Most frequent mutations causing FXI deficiency by ethnicity

Population	RefSeq	Genomic position*	Type of mutation	cDNA level†	Native protein	Mature protein	% of all mutated alleles in the analyzed population	Notes
Africans		Chr4:187 197 512	Missense	c.723C>G	p.F241L	p.F223L	23.5	Found 5 times in ExAC, 4 in Africans
East Asians		Chr4:187 201 251	Nonsense	c.841C>T	p.Q281X	p.Q263X	28.2	Found 11 times in ExAC, only in East Asians
		Chr4:187 206 808	Frameshift	c.1325delT	p.L442CfsX8	p.L424CfsX8	20.5	Found 8 times in ExAC, only in East Asians
Europeans	rs121965063	Chr4:187 195 347	Nonsense	c.403G>T	p.E135X	p.E117X	35.4	Type II mutation Found 95 times in ExAC, 85 in Europeans
	rs121965064	Chr4:187 201 412	Missense	c.901T>C	p.F301L	p.F283L	40.4	Type III mutation Found 100 times in ExAC, 97 in Europeans
Latinos		Chr4:187 205 398	Missense	c.1288G>A	p.A430T	p.A412T	25	Found 8 times in ExAC, 6 in Latinos
South Asians	rs121965063	Chr4:187 195 347	Nonsense	c.403G>T	p.E135X	p.E117X	12.5	Type II mutation Found 95 times in ExAC, 4 in South Asians
		Chr4:187 201 469	Frameshift	c.961_962delTG	p.C321HfsX37	p.C303HfsX37	12.5	Found 6 times in ExAC, 4 in South Asians

The most recurrent mutations (>10% of mutated alleles) were identified in the ExAC data set among all null mutations plus missense variants previously reported as associated with FXI deficiency in public databases (see "Methods").

*Numbering according to UCSC Genome Browser, human, February 2009 (GRCh37/hg19) assembly.

†Numbering starting from ATG according to NM_000128.

disease database as associated with FXI deficiency, only 56% of missense variants were predicted as potentially deleterious by 7 of 7 algorithms, leading to a different mutation distribution: 19.1% nonsense, 52.2% missense, 14% splicing, and 14.7% frameshift mutations (the distribution again was significantly different, though to a different extent; $\chi^2 = 9.7$; 2-tailed $P = .022$).

Based on ExAC data, we calculated exceptionally high prevalence rates, with values up to 29.3 in 10^6 and 16.5 in 10^6 among East Asians and Europeans (Table 1). We also tried to be more conservative, taking into account, among missense variants, only those already reported in the FXI-related literature/databases, and again the calculated prevalence was higher than expected (eg, 20.3 in 10^6 and 12.9 in 10^6 in East Asian and European populations) (Table 2). Our calculations could in theory even be an underestimate, considering that we applied a very conservative selection of deleterious variants (ie, we considered only missense changes predicted by 7 of 7 algorithms, as well as splicing defects affecting the invariant dinucleotides GT-AG). Indeed, on the basis of the observation that only half of the missense mutations listed in the FXI Deficiency Mutation Database would survive this selection, we can speculate that we could have "missed" a significant fraction of genetic defects responsible for reduced FXI levels. In addition, some variants may have been missed for technical problems related to exome sequencing, for example: (1) gross deletions and rearrangements may go undetected; (2) calling of indel mutations still represents a tricky step in exome analysis, so they can go unnoticed; and (3) mutations in the promoter region, and those located outside of the canonical splice sites, are not included by design.

Table 5. Comparison of constraint metrics for point mutations in F11 and F2 genes

Type of variant	Expected no. of variants	Observed no. of variants	Constraint metric*
Coagulation FXI (*F11*)†			
Synonymous	101.3	93	$z = 0.51$
Missense	210.6	229	$z = -0.62$
LoF	24.4	30	pLI = 0.00
Coagulation FII (prothrombin, *F2*)‡			
Synonymous	112.1	95	$z = 1.00$
Missense	251.0	164	$z = 2.69$
LoF	27.4	4	pLI = 0.96

LoF, loss of function.

*pLI indicates genes are classified in 3 classes according to their tolerance to LoF variants (completely tolerant, heterozygous LoF tolerated, heterozygous LoF not tolerated). pLI expresses the probability for a given gene to fall into the category of extremely intolerant to LoF. pLI ≥ 0.9 is attributed to extremely LoF-intolerant genes. *z* indicates deviation of observed counts from the expected ones. Positive *z* scores indicate intolerance to variation (ie, fewer variants than expected). Negative *z* scores indicate the observation of more variants than expected. For details, see http://exac.broadinstitute.org.

†Genomic coordinates: Chr4:187 187 099-187 210 835 (gene length, 23 736; 15 exons).

‡Genomic coordinates: Chr11:46 740 730-46 761 056 (gene length, 20 326; 14 exons).

Our data pose the question of why such surprisingly high prevalence rates of homozygotes/compound heterozygotes can be calculated for FXI deficiency. As also stated earlier, the most obvious possible reason is that its prevalence has always been underestimated because of the relatively mild symptomatology associated with the disease.[2] Another possibility could be related to a selective "advantage" conferred by FXI-null mutations to heterozygous or even homozygous carriers. Of note, Tucker and coworkers[23,24] observed evidence for disseminated intravascular coagulation after sepsis induced by cecal ligation and puncture in wild-type mice, but not in FXI knockout$^{-/-}$ mice; this survival advantage could be related to an alteration of the cytokine response to infection and to a reduced activation of the contact system.[25] On the other hand, it could be conceivable that we do not observe in the general population the predicted rates of homozygotes/compound heterozygotes because of possible defects during fetal implantation for FXI-deficient women/embryos, or because of problems associated with pregnancy. To discriminate between the "early lethality" vs "absence of bleeding" hypotheses, we compared the expected and actual frequency of all variants deposited in the ExAC server in *F11* and prothrombin (*F2*) genes. *F2* was chosen on the basis of its comparable size and exon-intron

structure with *F11*, and because its complete deficiency is considered to be incompatible with life.[26] Conversely to *F2*, this analysis showed a good tolerance of *F11* to loss-of-function mutations (Table 5), thus strongly supporting the hypothesis that the mildness of bleeding symptoms is the most likely explanation. Moreover, it has to be underlined that, unlike for women showing fibrinogen or factor XIII deficiency, the incidence of spontaneous miscarriage in women with FXI deficiency is similar to that of the general population.[27]

A potential limitation of this study is that our calculations were based on the assumptions that all mutations contribute equally to the phenotype, and that the analyzed populations are truly panmictic. Considering the first assumption, some of the identified FXI mutations have been shown to have a dominant-negative effect in the heterozygous state due to the dimeric structure of circulating FXI, leading to a dominant pattern of inheritance.[2] Therefore, the actual prevalence of moderate to severe FXI deficiency could be even higher than the one we calculated under the assumption of a "true" recessive disorder. Considering the assumption of random mating, it has to be kept in mind that >1 billion people live in societies where consanguineous marriages are accepted, and, worldwide, 15% of all newborns have consanguineous parents.[28] Consanguinity is a well-known factor able to significantly influence the prevalence rates for autosomal-recessive disorders.[29] Consanguinity rates vary greatly between and within countries, with highest prevalence in populations, like the ones that we analyzed, coming from North Africa, the Middle East, and South Asia.[28,30,31] This could result in "true" prevalence data even higher than the ones we calculated from allele frequencies.

A final consideration pertains to some outcomes of our analysis: the confirmation that, among ExAC populations, type II and type III mutations are virtually exclusive to Europeans, and the discovery of specific prevalent mutations in other populations (up to 28% of all mutated alleles; Table 4). This information, tentatively indicating the *F11* regions to prioritize for the molecular screening, could be of great value for diagnostic purposes, especially for countries where public health resources are scarce. Moreover, our results stress the notion that the availability of population-based genomic data will greatly improve our knowledge of the mutational spectrum as well as the prevalence of rare human genetic diseases.

Finally, recent evidence suggests the possible utility of FXI-targeted anticoagulants in treating atherosclerosis[32] and hypertension,[33] as shown in mouse-model studies, and in preventing thrombosis in patients undergoing knee arthroplasty.[34] These therapies, however, might not be suitable to FXI-deficient patients, and, before considering such treatments, measurement of FXI plasma levels should be recommended as FXI deficiency, besides the well-known high frequency among Ashkenazi Jews, is also unexpectedly frequent in other populations.

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Authorship

Contribution: All authors participated in the conception and design of the study, analysis and interpretation of data, and revision of the manuscript; R.A. conceived the study, wrote the manuscript, and contributed to the statistical analysis; E.M.P. was responsible for the statistical analysis of the data; V.R., F.P., M.M., and O.S. participated in the writing of the manuscript and critical review; and S.D. contributed to the writing of the manuscript and supervised the entire study.

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