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Factor 11 single-nucleotide variants in women with heavy menstrual bleeding

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ABSTRACT

In a previous study it was shown that lower factor XI (FXI) levels in women with heavy menstrual bleeding (HMB). Our aim was to determine the single-nucleotide variants (SNVs) in the *F11* gene in women with HMB. In addition, an extensive literature search was performed to determine the clinical significance of each SNV. Patients referred for HMB (PBAC-score >100) were included. With direct sequencing analysis of all 15 exons and flanking introns of the *F11* gene, 29 different non-structural SNVs were detected in 49 patients with HMB. Interestingly, most of these SNVs have previously been associated with venous thrombosis instead of bleeding. These findings have not helped to elucidate the molecular basis of HMB. They also question the specificity of previously reported *F11* variations in patients with thrombosis. More studies are needed to explain the lower FXI levels seen in patients with HMB.

IMPACT STATEMENT

- Women with mild deficiencies of factor XI (FXI) (< 70%) are prone to excessive bleeding during menstruation. Bleeding manifestations are not well correlated with plasma FXI levels and bleeding episodes can vary widely among patients with similar low FXI levels. In a previous study we showed that women with heavy menstrual bleeding (HMB) had normal, but on average, lower levels of FXI than controls.
- In light of these findings, we performed *F11* gene analysis to determine the single-nucleotide variants (SNVs) in women with HMB and performed an extensive literature search to determine the clinical significance of each SNV. By direct sequencing analysis of the *F11* gene we found 29 different non-structural SNVs in 49 women with heavy menstrual bleeding. Remarkably, a number of these SNVs have previously been implicated in thrombosis.
- These findings have not helped to elucidate the molecular basis of lower FXI levels in HMB. They also question the specificity of previously reported *F11* variations in patients with thrombosis. More studies are needed to explain the lower FXI levels seen in patients with HMB.

KEYWORDS

Heavy menstrual bleeding; factor XI; single-nucleotide variants

Introduction

Factor XI (FXI 2014) deficiency, also known as haemophilia C, is an autosomal bleeding disorder characterised by reduced plasma levels of FXI with a high prevalence (about 9%) in the Ashkenazi Jewish population (Bolton-Maggs 2009). The prevalence of FXI deficiency in Caucasians is reported as low, but might be underestimated (Mitchell et al. 2006; Zadra et al. 2008). Women with low levels of FXI (<70%) are prone to excessive bleeding during menstruation. However, bleeding manifestations are not well correlated with plasma FXI levels and bleeding episodes can vary widely among patients with similar low FXI levels (O'Connell 2003; Bolton-Maggs 2009). To date, more than 200 mutations have been reported (http://www.factorxi.org).

In plasma, FXI circulates as a homodimeric precursor of a serine protease (FXIa), which plays an essential role in the contact activation of coagulation through the conversion of FIX to FIXa in a calcium-dependent manner (Davie et al. 1991). Each FXI monomeric structure contains a heavy chain and a light chain that are joined together by disulphide bonds. The heavy chain contains four apple domains and the light chain contains the serine protease domain (Fujikawa et al. 1986). The fourth apple domain is necessary for dimerisation (Meijers et al. 1992).

The *F11* gene is located on the long arm of chromosome 4 (4q35) and contains 15 exons and 14 introns (Asakai et al. 1987). Exon 1 encodes the untranslated region (UTR), whereas the exon 2 encodes the signal peptide. All four apple domains are encoded in exons 3 to 10. Exons 11 to 15 encode the serine protease domain.

In our previous study (Knol et al. 2013), a 4% FXI deficiency (<70%) was found in unselected Dutch women with heavy menstrual bleeding (HMB). It was also found that

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Supplemental data for this article can be accessed here.

patients had significantly longer activated partial thromboplastin time compared to controls (26.5 vs 25.0 s; $p = .001$), despite higher levels of factor VIII. This turned out to be caused by lower median levels of FXI (100 vs 124%; $p < .001$).

In light of these findings, *F11* gene analysis was performed to determine the single-nucleotide variants (SNVs) in women with HMB. In addition, an extensive literature search was performed to determine the clinical relevance of the identified SNVs.

Materials and methods

Inclusion

Patients referred for heavy, regular menstrual periods were included. Exclusion criteria were Pictorial Blood loss Assessment Chart-score <100 (Higham et al. 1990; Janssen et al. 1998), known bleeding disorder, use of any intrauterine device within 2 months prior to inclusion and treatment with antifibrinolytics, anticoagulants, non-steroidal anti-inflammatory agents, progestagens or combined oral contraceptives. Eligible women were asked to fill out a structured questionnaire for medical, obstetrical and gynaecological history, including all items of the Tosetto bleeding score (Rodeghiero et al. 2007). Eligible women were invited to our clinic and had a gynaecological examination and pelvic ultrasonography in the first week after the menstruation. The study was approved by the Institutional Review Board of the University Medical Center of Groningen. Informed consent was obtained from all patients.

Blood collection and sample preparation

Venous blood was taken from all patients in the first week after the menstruation. The blood samples were taken before the gynaecological examination. Blood samples were anticoagulated with a 1:10 volume of 0.109 M trisodium citrate. Platelet-poor plasma was prepared by centrifugation at 2500×*g* for 15 min, aliquoted and immediately frozen at −80 °C, and analysed after rapid thawing at 37 °C. Genomic DNA was obtained from peripheral blood samples using the Qiacube system.

FXI assay

FXI activity levels were determined by a one-stage clotting assay (Siemens, Marburg, Germany). Reference interval was 65–150%.

PCR amplification and sequencing

PCRs were performed for each of the 15 exons and flanking introns. Primers are indicated in Table 1. Reactions (25 µl) for all PCRs except for exon 9 and 14 contained 1 µl of primer (20 pmol), 1.6 µl of $MgCl_2$ (25 mM), 0.3 µl of dNTPs (25 mM), 0.4 µl of Faststart Taq (5Ul/µl), 5 µl of 5× GC-rich solution, 2.5 µl of 10× PCR-buffer (–$MgCl_2$), 11.2 µl of DEPC H_20 and 2 µl of DNA (10 ng). For exons 9 and 14, 1 µl instead of 1.6 µl of $MgCl_2$ (25 mM) was used. The PCR protocol for exons 9 and 14 started with denaturation for 5 min at 94 °C, followed by 40 cycles at 94 °C for 30 s, 55 °C for 30 s and 70 °C for 1 min. The cycling protocol of the remaining exons was almost the same, only the annealing temperature was 49 °C instead of 55 °C, and the amount of cycles was 35 instead of 40. M13-tailed primers enabled the use of standardised amplification conditions for the sequencing PCR (96 °C for 1 min, 30 cycles at 96 °C for 30 s, 55 °C for 15 s and 60 °C for 2 min). The sequencing PCR was performed on both strands using Big Dye terminators V1.1 and an ABI 3130xl analyser. The chromatograms were analysed with CLC main workbench.

Table 1. Primers.

	Primer sequence (5′ > 3′)	Position
Exon 1F	CCAAAGTCTCCTCCCTCCAT	−130 to −110
Exon 1R	TGTTGTTCCACATTTCCTCTCA	169 to 191
Exon 2F	AGAAGGATCTGAACACAGAGAGC	−299 to −276
Exon 2R	CCTGCTTTGAAAACCTCTGG	189 to 209
Exon 3F	GTTTCCACCTGAGGCTGTTC	−181 to −161
Exon 3R	TTGCGTAAACACCATCACTTC	145 to 166
Exon 4F	CAGTCAGGCTAAACAGAAACACACAGA	−286 to −263
Exon 4R	GGGGGTTTATTTCCACATAGC	92 to 113
Exon 5F	CTGAGGAAAGGTGGGTGAAA	−110 to −90
Exon 5R	GCGCTTTTGAAATTAGCCAAG	231 to 252
Exon 6F	TGAGAAGGGCTTGAGAAGTCA	−272 to −251
Exon 6R	TCCGTTTCATCGTGAGCATA	81 to 101
Exon 7F	ACCAGCTTATGCTCACGATG	−260 to −240
Exon 7R	GAAAGGGAGAGGGGCTAGAA	128 to 148
Exon 8-9F	ACCTAAGGGCCATGGAGTGT	−191 to −171
Exon 8-9R	CATTGGTGCACAGTTTCTGG	84 to 104
Exon 9-10F	TGCTGTCTAGTGTTCTGCCATT	−10 to 11
Exon 9-10R	TGGTCAGCTTGAGTGACAGG	142 to 162
Exon 11F	ATGTTTTGCTTTGGCAGCTT	−158 to −138
Exon 11R	GCCTTGTACCTGCACCTGTT	176 to 196
Exon 12F	TGTCCATCATTGGCAGAAAA	−113 to −93
Exon 12R	AGCCAGGAAAGTGTGTCAGC	154 to 174
Exon 13F	GCAACTTGTGCAGGATCAAA	−256 to −236
Exon 13R	TTGGGTGATTTTTGCCTCTC	133 to 153
Exon 14F	ATGGTTATTCTACAAACGAACCAAA	−39 to −14
Exon 14R	TCCTTGCTTTTGATTTCAGTCTAAG	451 to 476
Exon 15F	AAGACAACATTTTAGGCAAAATCAG	−100 to −75
Exon 15R	TTTCTTCCAGTTTTCATCTTTCATC	207 to 232
M13F	TGTAAAACGACGGCCAGT	na
M13R	CAGGAAACAGCTATGACC	na

Exon 9–10 F is partly bound within exon 9.

Nomenclature

The nomenclature was according to the guidelines of the Human Genome Variation Society. For cDNA numbering, nucleotide 1 is the A of the ATG translation initiation codon (c.1). For amino acid numbering, the ATG initiation codon corresponds to the first amino acid (p.1).

Literature search

To determine the clinical significance of each SNV, a search in PubMed was performed to identify all relevant references for each SNV. More specifically, references were included if a significant clinical association was reported, if molecular genetic analysis was performed to identify causal *F11* gene defects, if structural features of a specific variant were analysed, or when haplotype analysis was performed with specific variants. Based on these criteria, references were categorised in association studies and molecular analysis.

Table 2. Patient characteristics.

	Patients ($n = 49$)
Age in years, median (range)	44 (26 to 54)
PBAC-score in points, median (Q1–Q3)	272 (204 to 557)
FXI level in %, median (Q1–Q3)	96 (88 to 118)
FXI level <100%, *n* (%)	26 (53)
Tosetto bleeding score in points, median (range)	0 (−2 to 7)

PBAC: Pictorial Blood loss Assessment Chart; FXI: Factor XI.

A reference search was performed starting with the SNV reference id (rs-code). To identify rs-codes, a selection of the *F11* gene sequence containing the base pair change was paste in mutationtaster (http://mutationtaster.org/) and a search was performed using NCBI gene ID 2160 and Ensembl transcript ID ENST0000403665. If no references were recovered for the rs-codes, additional keywords such as base pair change (c.), amino acid change (p.), gene name, FXI deficiency, polymorphism, association studies or GWAS were used. In addition, each reference including supplementary data was screened for additional SNVs. Finally, it is worth noting that despite using the above mentioned keywords no references may be retrieved. This may be caused by the following: (1) in several publications the coding sequence numbering is according to Fujikawa, i.e. −43 bp (Fujikawa et al. 1986); (2) some SNVs such as rs4253398 and rs3822057 have been given a different base change position, i.e. −231 T > C, and −138 C > A, respectively; (3) most amino acid changes are indicated after the signal peptide is cleaved (−18).

Results

49 patients were included with a median age of 44 years (range: 26–54). Median FXI level was 96% (range 61%–155%); vs 124% in our previously published menstrual cycle-matched controls (Knol et al. 2013). In 53% of the patients, the FXI level was <100% (Table 2). In 2 patients FXI levels below the normal range of 65% were found. One patient (number 46 in the supplementary table S1) had a level of 61% factor XI, which was in the normal range (83%) when the measurement was repeated and one patient had a FXI of 64%, which was not repeated. None of these 49 patients had a history of deep venous thrombosis. In total, 29 different non-structural SNVs were identified in 49 patients (Table 3).

Non-synonymous variants

Two non-synonymous variants were identified: rs5969 (p.Gln244Arg) and rs202061241 (p.Val615Met). Rs5969 is the result of an A to G substitution at nucleotide 731 in exon 7. This mutation was first described by Martincic et al. (Martincic et al. 1998; Erratum 1999). This paper reports on the genetic analysis of *F11* gene in two African–American patients with mild FXI deficiency and one patient of European Jewish ancestry with severe FXI deficiency (patient 3). This last patient was included in the study as an abnormal control. The propositus, a 9-year-old boy with a history of excessive bleeding and mild FXI deficiency, was compound heterozygous for rs5969 and rs145168351 (p.Ser266Asn). His mother was heterozygous for rs145168351, and also experienced excessive bleeding. Besides being compound heterozygous for type II (rs121965063, p.Glu135Ter) and type III (rs121965064, p.Phe301Leu) variants, the abnormal control did not contain the other two non-synonymous variants. The binding affinity between FXI and FIX (Km) differed considerably from wild-type FXI (Sun et al. 2001). However, rs5969 was associated with FXI activity levels comparable to wild-type when tested in an APTT-based assay (Martincic et al. 1998). In line with this, the catalytic efficiency (*k*cat) for FIX activation was shown to be comparable to that of wild-type FXI, thus normalising the APTT result (Sun et al. 2001). Various clinical studies as well as studies that examined the structural features of rs5969 have confirmed the minimal effect (Mitchell et al. 1999; Mitchell et al. 2003; O'Connell et al. 2005; Mitchell et al. 2006; Saunders et al. 2009). Therefore, our findings were in accordance with the normal FXI level (83%) was found in our patient. Rs202061241 is caused by a G to A substitution at nucleotide 1843 in exon 15, which is located in the protease domain. To our knowledge, no clinical data on this mutation has been reported. Therefore, PROVEAN (2014) (http://provean.jcvi.org/index.php) was used to determine the functional effects of this mutation. With a score of −0.276, this mutation was said to be neutral (default threshold −2.5). This finding supports the FXI level (93%) found in this patient.

Synonymous variants

Our study detected six synonymous variants. One patient (FXI level: 96%) was heterozygous for a SNV in exon 5 (c.423 G > A), maintaining the threonine at amino acid position 141 in the second apple domain (p.Thr141=). This variant had no reference ID, though it was found in the Exome Aggregation Consortium (Exac) Browser (http://exac.broadinstitute.org/), with an allele frequency of 0.000008243.

A missense mutation at this position, resulting in a threonine to methionine substitution has been described in combination with the nonsense mutation p.Glu135Ter in a severe FXI-deficient patient from the Abruzzo region in Italy (Castaman et al. 2008).

The five other synonymous variants (rs5973, rs5974, rs5970, rs5971 and rs5976) have been extensively used as markers for haplotype analysis (Bolton-Maggs et al. 2004; Quelin et al. 2004; Zadra et al. 2004; Zadra et al. 2008; Kim et al. 2012; Bicocchi et al. 2013), which is not surprising as three (rs5973, rs5974 and rs5970) are in marked linkage disequilibrium with one another (Tarumi et al. 2000). These three neutral variants were initially reported by Martincic et al. (1998).

Non-coding variants

Twenty-one SNVs were located in the non-coding regions of the *F11* gene. Based on the literature search (Table 3), these variants have mostly been mentioned in the context of the risk of venous thrombosis. Among these variants, rs2289252 was most frequently reported to be independently associated with venous thrombosis. Furthermore, rs2289252 was associated with miscarriages, decreased APTT and high FXI levels.

Table 3. Single-nucleotide variants.

				Association studies			Molecular analysis	
dbsnp	Base change	AA change	Region	Trait	Association	Reference	Causal	Reference
rs771153790	c.423G > A	p. Thr141=	Exon 5	NR	NR	NR	NR	NR
rs5973	c.429C > T	p.Asp143=	Exon 5	VTE	N	(Gerdes et al. 2004)	N	(Martincic et al. 1998)[a], (Ventura et al. 2000; Zivelin et al. 2002; Bezak et al. 2005; Zadra et al. 2008; Bicocchi et al. 2013)
rs5969	c.731A > G	p.Gln244Arg	Exon 7	NR	NR	NR	N	(Martincic et al. 1998)[a], (Mitchell et al. 1999; Sun et al. 2001; Mitchell et al. 2003; O'Connell et al. 2005; Mitchell et al. 2006; Saunders et al. 2009)
rs5974	c.801A > G	p.Thr267=	Exon 8	VTE	N	(Gerdes et al. 2004; Bezemer et al. 2008)	N	(Martincic et al. 1998)[a],(Ventura et al. 2000; Zivelin et al. 2002; Zadra et al. 2004; Zadra et al. 2008; Kim et al. 2012; Bicocchi et al. 2013)
rs5970	c.1191T > C	p.Gly397=	Exon 11	VTE	N	(Gerdes et al. 2004)	N	(Zivelin et al. 2002; de Moerloose et al. 2004; Zadra et al. 2004; Bezak et al. 2005; Jayandharan et al. 2005; Zadra et al. 2008; Kim et al. 2012; Bicocchi et al. 2013), (Martincic et al. 1998)[a]
rs5971	c.1812G > T	p.Arg604=	Exon 15	VTE	N	(Gerdes et al. 2004; Bezemer et al. 2008; Germain et al. 2011; Morange et al. 2011)	N	(Quelin et al. 2004; Zadra et al. 2004, 2008; Fardesfahani et al. 2008; Kim et al. 2012; Bicocchi et al. 2013)
rs5976	c.1839G > A	p.Glu613=	Exon 15	VTE	N	(Gerdes et al. 2004)	N	(Zivelin et al. 2002; Quelin et al. 2004; Zadra et al. 2004; Zadra et al. 2008; Bicocchi et al. 2013)
rs202061241	c.1843G > A	p.Val615Met	Exon 15	NR	NR	NR	NR	NR
rs3733403	c.-316C > G		UTR 5	VTE/Stroke	N and?/Y	(Bezemer et al. 2008; Reiner et al. 2009; Hanson et al. 2013)	?	(Tarumi et al. 2003; Quelin et al. 2006)
rs3822056	c.-446G > T		nearGene-5	VTE/APTT	N and?/N	(Bezemer et al. 2008; Morange et al. 2011)	?	(Tarumi et al. 2002, 2003; Quelin et al. 2006)
rs925451	c.-2 + 120G > A		Intron 1	VTE/HDP/ APTT/Stroke	Y and N/?/Y/Y	(Sato et al. 2006; Li et al. 2009; Delluc et al. 2010; Germain et al. 2011; Morange et al. 2011; Hanson et al. 2013)	N	(Quelin et al. 2004)
rs4253398	c.-1-229T > C		Intron 1	NR	NR	NR	N	(Ventura et al. 2000; Zivelin et al. 2002; Bolton-Maggs et al. 2004; Zadra et al. 2004; Zadra et al. 2008; Kim et al. 2012)
rs4253399	c.-1-196T > G		Intron 1	VTE/APTT/FXI	Y and N/?/?	(Li et al. 2009; Sabater-Lleal et al. 2012; Tang et al. 2013)	N	(Kim et al. 2012)
rs3841991	c.-1-148_-1-147insAT		Intron 1	NR	NR	NR	NR	NR
rs3822057	c.-1-138A > C		Intron 1	VTE	Y, N, and?	(Smith et al. 2007; Li et al. 2009; Arellano et al. 2010; de Haan et al. 2012; van Hylckama Vlieg et al. 2014)	N	(Zivelin et al., 2002; Bolton-Maggs et al. 2004; Zadra et al. 2004, 2008; Jayandharan et al., 2005; Kim et al. 2012), (Ventura et al. 2000)[a]
rs1593	c.485 + 122T > A		Intron 5	VTE/APTT/Stroke	N and?/Y/Y	(Bezemer et al. 2008; Li et al. 2009; Dahm et al. 2012; Tang et al. 2012; Hanson et al. 2013)	NR	NR
rs4253410	c.485 + 181T > C		Intron 5	NR	NR	NR	NR	NR
rs4253414	c.486-88T > C		Intron 5	VTE	N	(Bezemer et al. 2008)	NR	NR

(*continued*)

Table 3. Continued

				Association studies			Molecular analysis	
dbsnp	Base change	AA change	Region	Trait	Association	Reference	Causal	Reference
rs4253840	c.486-181C > T		Intron 5	NR	NR	NR	NR	NR
rs116667976	c.1304 + 12G > A		Intron 11	NR	NR	NR	NR	NR
rs2289251	c.1481-215C > T		Intron 12	NR	NR	NR	NR	NR
rs2289252	c.1481-188C > T		Intron 12	(Recurrent) VTE/ APTT/FXI/ Miscarriage	Y, N and?/Y/Y/N	(Smith et al. 2007; Reiner et al. 2009; Li et al. 2009; Smith et al. 2009; Delluc et al. 2010; Arellano et al. 2010; Dahm et al. 2012; de Haan et al. 2012; Lunghi et al. 2012; Tang et al. 2012; El-Galaly et al. 2013; van Hylckama Vlieg et al. 2014; Rovite et al. 2014; Sokol et al. 2014; Bruzelius et al. 2015[a,b])	NR	NR
rs2289253	c.1481-34G > T		Intron 12	NR	NR	NR	N	(Kim et al. 2012)
rs2289254	c.1576 + 51C > A		Intron 12	NR	NR	NR	NR	NR
rs4253427	c.1716 + 248G > A		Intron 14	NR	NR	NR	NR	NR
rs4253428	c.1716 + 250G > A		Intron 14	NR	NR	NR	NR	NR
rs74536324	c.1716 + 252C > T		Intron 14	NR	NR	NR	NR	NR
rs4253429	c.*265A > G		UTR 3	NR	NR	NR	NR	NR
rs4253430	c.*296G > C		UTR 3	VTE	N and?	(Bezemer et al. 2008; Li et al. 2009)	NR	NR

NR: not reported; associations are indicated by Y: significant, N: non-significant or?: doubtful; VTE: venous thromboembolus; APTT: activated partial thromboplastin time; HDP: hypertensive disorders in pregnancy; FXI: factor XI.
[a]The first report on this single-nucleotide variant.

This SNV is located in intron 12 (c.1481–188) and the result of a C > T substitution. Eight other SNVs, including rs1593, rs3822057, rs925451, rs4253430, rs4253414, rs4253399, rs3733403 and rs3822056 also showed an association with venous thrombosis. Interestingly, all these SNVs are in linkage disequilibrium with rs2289252 (SNAP, 1000 genomes, Bioinformatics).

It is tempting to speculate that an overexpression of these variants could compensate for low FXI level in these patients and may reset the haemostatic balance to a less-haemophilic phenotype. To test this hypothesis, the allele frequencies of all SNVs between high (> 100%) and low (<100%) FXI levels were compared using the Fisher Exact probability test in a 2 × 3 contingence table. A p value of less than .05 indicated significance. No significant difference was observed between both the groups. Moreover, even when the lowest percentile was taken and compared to the rest this difference remained non-significant.

Two identified SNVs in high LD (rs373403 and rs3822056) are located in the promotor region of the *F11* gene. In our cohort, nine patients had one or both of these SNVs with a median FXI level of 96% (range 61%–129%). Rs373403, a variant that is caused by a C to G base change at c.-316, has been shown to negatively affect the transcription binding (Tarumi et al. 2003). However, the overall effect is probably low, because a region between 381 and 363 bp upstream of exon 1, is responsible for maximum promotor activity in HepG2 hepatocellular carcinoma cells (Tarumi et al. 2002). This region contains the sequence ACTTTG that has been identified in several gene promotors of coagulation factors for being the binding site for transcription factor hepatocyte nuclear factor 4 (HNF4) (Reijnen et al. 1992; Erdmann and Heim 1995; Hung and High 1996). Also, no variant was located at the binding site for miR-181a-5p, a microRNA which is inversely correlated with *F11* mRNA levels (Salloum-Asfar et al. 2014).

Discussion

This study was performed because it was shown (Knol et al. 2013) that women with HMB had lower mean FXI levels than menstrual cycle-matched controls. 29 different non-structural SNVs were detected in 49 patients with HMB.

Additionally, the SNVs found in our patient group were also compared with the literature. Literature shows that *F11* gene analysis is mainly carried out in the context of the risk of deep venous thrombosis and elevated levels of FXI. Most SNVs that were found are therefore already described in relationship with either venous thrombo-embolism, stroke, decreased APTT, hypertensive disorders in pregnancy, higher FXI levels or miscarriages. Remarkably, our results show that these SNVs are also present in women with HMB and thus in a group with increased bleeding tendency. Noteworthy is that none of the women in our study had a previous VTE. This result directly undermines the assumed relationship between these SNVs and venous thrombosis.

A limitation of our study is that the patient group is small. As a consequence it was impossible to provide haplotype data and multiple comparisons could not be made. Nonetheless, this is the first study which gives an overview of the molecular background of the *F11* gene, as found in patients with HMB. Therefore, our group can serve as a reference group for future studies. Another limitation is that it

was not possible to compare the patients with a control group of women with a normal amount of blood loss during their menstruation. However, it was possible to compare the FXI levels of our patients with a control group from our previous study (Knol et al. 2013). In addition, because most of the SNVs that were found have previously been described, an extensive literature search was performed to determine the clinical significance of each SNV.

Conclusions

By direct sequencing analysis of the *F11* gene, 29 different non-structural SNVs were found in 49 women with HMB. These findings have not helped to elucidate the molecular basis of HMB. They also question the specificity of previously reported *F11* variations in patients with thrombosis. More studies are needed to explain the lower FXI levels seen in patients with HMB.

Disclosure statement

K.M. reports grants and other from Baxter, Bayer and Sanquin; other from Pfizer and Boehringer Ingelheim, outside the submitted work. S.W.-V. reports travel support from CSL Behring, outside the submitted work. The remaining authors have nothing to disclose. The authors alone are responsible for the content and writing of the paper.

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