Pathogenicity analysis of variations and prenatal diagnosis in a hereditary coagulation factor XIII deficiency family

Liwei Sun\(^a\), Qijiang Yan\(^b\), Yonghua Wang\(^a\), Hualei Luo\(^a\), Peng Du\(^a\), Reem Hassan\(^a\), Li Liu\(^c\) and Weiying Jiang\(^a\)

\(^a\)Department of Medical Genetics, ZhongShan School of Medicine, Sun Yat-sen University, Guangzhou, People’s Republic of China; \(^b\)Guangzhou Kingmed Diagnostics Technology Co., LTD, Guangzhou, People’s Republic of China; \(^c\)Guangzhou Women and Children’s Medical Center, Guangzhou, People’s Republic of China

ABSTRACT

Objectives: Prenatal diagnosis (PND) procedure is urgent to be established for timely management and fatal consequence prevention of factor XIII deficiency (FXIIID), and variations data among Chinese are very scanty. We aimed to find a novel mutation among Chinese and establish a rapid and precise PND procedure with pathogenicity analysis to contribute to the prevention of postpartum hemorrhage in pregnant women and central nervous system bleeding in newborns.

Methods: FXIIID was diagnosed by qualitative and quantitative tests of clot solubility test and enzyme-linked immunosorbent assay, respectively. Variations were detected by direct sequencing of F13A and F13B genes in the pedigree and the unborn fetus. Pathogenicity assessment of variations was based on American College of Medical Genetics and Genomics Guidelines.

Results: Ten variants in the F13A gene including a novel missense mutation in exon 10, a nonsense mutation in exon 4, a missense mutation in exon 12, 2 missense mutations in exon 14, 3 polymorphisms in intron 10, 2 polymorphisms in intron 14 were detected. Two variants in the F13B gene including a polymorphism in 3’UTR and a synonymous mutation were detected. The compound heterozygous mutations of the nonsense mutation and a novel missense mutation of the F13A gene caused the deficiency in proband, and the fetus which was evaluated to be unaffected by PND was born successfully and the results were verified by follow-up visits.

Discussion: We first established the PND procedure with pathogenicity assessment in FXIIID patients. The F13A gene mutations’ spectrum of the Chinese Han population was enriched.

Introduction

Factor XIII (FXIII) is a heterotetrameric protein complex composed of two catalytic subunits (FXIII-A\(_2\)) and two carrier subunits (FXIII-B\(_2\)) in plasma. Hereditary FXIII deficiency (FXIIID) is an autosomal recessive disorder with an extremely rare incidence of one per two million worldwide [1]. It is characterized by life-threatening bleeding episodes, including intracranial hemorrhage (ICH), recurrent pregnancy loss (RPL) and neonatal umbilical cord bleeding [2].

Disease-causing mutations of the F13A gene or F13B gene are responsible for FXIIID. The F13A gene is located on chromosome 6p24-25 and contains 15 exons and 14 introns; it encodes a 731-amino acid protein which consists of five structural domains: an activation peptide (residues 1–37), \(\beta\)-sandwich (residues 38–183), catalytic core region (residues 184–515) which contains the catalytic triad of Cys314–His373–Asp396 [3], \(\beta\)-barrel 1 and 2 (residues 516–627 and residues 628–731, respectively) [4]. To date, 183 F13A gene mutations had been reported in The Human Gene Mutation Database (HGMD) with a high degree of ethnic heterogeneity; only four missense mutations [5–7], four nonsense mutations [6,8], three deletions [9,10] of them were detected among Chinese. The F13B gene, located on 1q31.3, encodes the B subunit of FXIII-B\(_2\), which likely act as carrier proteins. Until March 2017, 20 disease-causing mutations had been reported in HGMD, including 10 missense mutations’3 splicing mutations’4 small deletions mutations and 3 small insertion mutations.

Carrying out prenatal diagnosis (PND) in limited time contributes crucially to providing timely management to decrease the risk of maternal and neonatal fatal consequences and enable an optimal delivery. A pregnant woman with FXIIID is at an increased risk of miscarriage and postpartum hemorrhage (PPH), and so is her affected fetus at increased risk of neonatal umbilical cord and central nervous system (CNS) bleeding [11,12]. There are only six cases of PND for FXIIID families reported [8,13–17]. PND based on polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of population-specific pathogenic
mutation has been proposed in Iran [17]. However, it is inapplicable to most of the population without recurrent mutations; especially the functional verification experiments for novel mutations are improper due to time constraint. Hence, it is urgent to establish a rapid and precise PND procedure with pathogenicity assessment.

Our study for the first time established a rapid and precise PND procedure with pathogenicity analysis based on American College of Medical Genetics and Genomics (ACMG) guidelines. We also reported a novel missense mutation which can enrich the F13A gene mutations’ spectrum and contributed to further study on genotype–phenotype correlation.

Materials and methods

Patients

The proband was a 32-year-old pregnant woman, who presented frequently with subcutaneous bleeding, poor wound healing and had suffered from ICH and corpus luteum rupture, received transfusion of fresh frozen plasma (FFP) monthly. A total of five individuals of two generations of the family participated in our research (Figure 1). No bleeding history was found in other family members. Considering the prolonged half-life of FXIII, the samples of the proband receiving FFP had been taken at least 3 weeks after the last treatment in case the results were affected. Ethical approval for this study was obtained from the Sun Yat-sen University Ethics Committee. Informed consent for participation in the study was obtained from all individuals.

Methods

Qualitative diagnosis of FXIII deficiency

We diagnosed the deficiency by the following steps: (1) took precise clinical characteristics of patient and family history; (2) performed routine coagulation tests including prothrombin time, activated partial thromboplastin time, thrombin time and fibrinogen (FIB) on an STA-R analyzer (Diagnostica Stago, Asnieres, France) of the family; (3) performed clot solubility test (urea/calcium method); the clot solution was evaluated at 15 minutes, 1 hour and 24 hour intervals. In cases with abnormal clot solubility test results, a mixing study was performed to distinguish the presence of the FXIII inhibitor from congenital FXIII deficiency [18,19].

Quantitative measurement of FXIII

Quantitative measurements of FXIII were determined using the Abcams Factor XIII Human in vitro enzyme-linked immunosorbent assay (ELISA) kit (Cambridge, UK). The absorbance was read on the Sunrise™ Absorbance Reader instrument (Tecan, Austria), the standard curve was generated by using the MasterPlex 2010 software (www.Masterplex.co.uk/index.html), the graph was plotted using the standard concentrations on the x-axis (0, 2.5, 5, 10, 20, 40, 80, 160 ng/ml) and the corresponding means at 450 nm absorbance on the y-axis. All measurements were performed in duplicate and the unknown sample concentration determined from the standard curve and the value multiplied by the dilution factor (1:1000).

RNA isolation and Quantitative real-time RT–PCR

Total RNA was isolated and purified from blood of the family and healthy controls using the QIAamp RNA Blood Mini Kit (Qiagen, Germany). The quality and quantity of the obtained RNA were determined using a spectrophotometer and 2% agarose gel electrophoresis. Potential genomic DNA contamination was excluded using a PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, Dalian, China). The cDNA was generated by reverse transcription of total RNA using a PrimeScript™ RT reagent Kit (Takara, Dalian, China). Quantitative real-time RT–PCR was performed on Bio-Rad CFX96 Touch™ (Bio-Rad Laboratories, Inc., 2000 Alfred Nobel Dr. Hercules, CA 94547, USA). The mRNA expression was defined as fold change from the human glyceraldehyde-3-phosphate dehydrogenase gene expression level by calculating as (2−ΔΔCT).

Molecular genetic analyses

Genomic DNA was extracted from peripheral whole blood using a Qiagen DNA extraction kit (Qiagen, Hilden, Germany). The quality and quantity of obtained DNA were evaluated by Nanodrop 2000c (Thermo Scientific, USA). All the exons along with intron–exon boundaries and 5′,3′–untranslated regions (UTR) of the F13A gene and F13B gene were amplified by PCR on ABI Veriti™ Thermal Cycler (Applied Biosystems, USA) with primers described previously (see lists of primers of the F13A gene and F13B gene at http://www.f13-database.de). PCR products were electrophoresed on 2% agarose gels to confirm amplification
of right-sized fragments before being purified and sequenced on ABI PRISM 3700 (Applied Biosystems, Foster city, CA, USA).

**Pathogenicity assessment**

The variations are classified into five categories based on the standard and guidelines for the interpretation of sequence variants by ACMG [20]: (1) pathogenic, (2) likely pathogenic, (3) uncertain significance, (4) likely benign and (5) benign.

Pathogenicity assessment was performed as the following steps: (1) Databases’ searching: Check databases including Human Genome Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/), disease-specific databases as FXIII Registry Database (http://www.f13database.de/) as well as the published literature. Classify it as a novel mutation if it has not been detected; (2) Segregation data: The segregation of the variant in all family members was performed; (3) Population data: Population databases including 1000 Genomes (http://browser.1000genomes.org), Exome Variant Server (http://evs.gs.washington.edu/EVS), Exome Aggregation Consortium (http://exac.broadinstitute.org/) are used to obtain the frequencies of variants among populations. Assessed novel missense variants by analysis of at least 100 unrelated normal subjects; (4) Conservation analysis: Sequence alignment was performed by the Clustal X Software (www.clustal.org) for conservation analysis; (5) Pathogenicity prediction tools: Multiple bioinformatics software are employed, such as Polymorphism Phenotyping-2 (PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/), Sorting Intolerant From Tolerant (SIFT, http://sift.jcvi.org/), Align GVGD (http://agvgd.hci.utah.edu/index.php), Protein Variation Effect Analyzer (PROVEAN, http://provean.jcvi.org/index.php). Tools including GeneSplicer (http://ccb.jhu.edu/software/genesplicer/), Human Splicing Finder (http://www.umd.be/HSF) were used to evaluate splice site variants, then it was confirmed by RT–PCR followed by sequencing; (6) Structural analysis: The crystallographic model of the recombinant human cellular FXIIIA zymogen (EC:2.3.2.13) was downloaded from the Protein Data Bank (www.rcsb.org/pdb/; PDB-ID:1F13) and impact of an amino acid substitution on the structure and function of the protein was analyzed using PyMol software (DeLano Scientific, San Carlos, CA, USA) (http://www.pymol.org/) which can visualize the position and molecular surroundings within the FXIII-A2 homodimer. Surface area interface residues, the residues contributing to electrostatic and hydrophobic interactions were determined with PDBePISA (http://www.ebi.ac.uk/msd-srv/prot_int/pistart.html).

**Prenatal diagnosis**

Then amniocentesis was performed at 19 weeks of gestation and fetal DNA was extracted from amniotic fluid using the TIANamp Micro DNA Kit (Tiangen, Beijing, China), and then DNA sequencing was performed to detect the disease-causing mutations.

**Management**

In order to avoid bleeding complications, the administration of FXIIIA at a dose of 250 IU weekly early in pregnancy until 23rd week, and increased thereafter to 500 IU per week, then increased to the dose of 1000 IU during labor and delivery widely were recommended [21]. However, cryoprecipitate was used to achieve the recommended management during pregnancy due to the lack of access to FXIII concentrate in this case. 2 units of cryoprecipitate per week were continued and were increased to 3 units at 23 weeks’ gestation, then a booster dose of 6 units was given at the 38th week of gestation during labor and delivery.

**Results**

Routine coagulation tests revealed normal ranges within the pedigree. Qualitative diagnosis by clot solubility test showed that the clot was mostly dissolved within 30 minutes, and became insoluble when normal plasma was mixed with patient's plasma which indicated FXIII-D. The four-parameter logistic curve was generated \(y = (a-d)/(1 + (x/c)^b) + d, a = 0.003, b = 0.63439, c = 27.22574, d = 2.68333\) and showed no detectable FXIII in proband which suggests rapid degradation of the faulty protein (Table 1). The FXIIIa mRNA expression of the proband was significantly decreased to levels below the threshold which leads to the severe phenotype (Figure 2).

Molecular diagnosis by full gene sequencing of the F13A gene and F13B gene was performed to confirm our diagnosis. Ten mutations in the F13A gene including 1 nonsense mutation in exon 4 (c.523C>T, p.Arg174X), 1 novel missense mutation in exon 10 (c.1226G>C, p.Arg408Pro), 1 missense mutation in exon 12 (c.1694C>T, p.Pro564Leu), 2 missense mutations in exon 14 (c.1951G>A, c.5978T>G, rs5978/ c.1305 + 104G>A, rs2274391), 2 polymorphisms in intron 14 (c.1909−29G>C, c.2274394/c.2045 + 39G>A, rs5980) were detected. A polymorphism in 3’UTR (c.*143G>A, rs698859) and 1 synonymous mutation (c.1806T>C, rs5998) in the F13B gene were detected.

**Table 1.** The ELISA analysis for FXIII level in the pedigree.

| FXIII level (mg/l) | Relative FXIII level % |
|-------------------|------------------------|
| Proband (II:2)    | 0.41                   | 0                     |
| Proband’s father (I:1) | 14.26               | 69.5                  |
| Proband’s mother (I:2) | 9.94                | 47.6                  |
| Standard plasma   | 20.87                  | 100                   |
The genotypes’ information of the F13A gene and F13B gene in all family members was summarized (Table 2). Representative chromatograms from Sanger sequencing of the F13A gene and F13B gene are shown (Figure 3).

The nonsense mutation in exon 4 (c.523C > T, p. Arg174X) reported among Chinese population was classified as a pathogenic variation. The F13A gene was reported to be highly polymorphic where many polymorphisms have been reported, including Val34Leu (c.103G > T; rs5985) in exon 2, Tyr204Phe (c.614A > T; rs3024477) in exon 5, Pro564 Leu (c.1694C > T; rs5982) in exon 12, Val650Ile (c.1951G > A; rs5987) and Glu651Gln (c.1954G > C; rs5988) in exon 14 [22]. In our study, we detected Pro564 Leu, Val650Ile and Glu651Gln and their allele frequencies all exceeded 1% among the population (21.6, 6.759, 20.87%, respectively) according to Exome Aggregation Consortium; so we classified them as polymorphisms which are likely benign. The c.1226G > A (p. Arg408Gln) in exon 10 of the F13A gene had been detected in England, Tunis and India [23–26]. For the first time, we found c.1226G > C (p. Arg408Pro). Homologous sequence results showed that the Arg408 was highly conserved among homologous species (Figure 4). Bioinformatics software predicted it as a deleterious mutation (Table 3). The analyzing model demonstrated Arg408 formed hydrogen bond interactions with Arg260. The salt bridge between Arg260 amino acid in the catalytic core domain of one FXIII A monomer and Asp404 in catalytic core domain of another monomer is crucial in the FXIII-A2 homodimer formation [27] and introduction of a positively charged arginine in the position would probably destroy their

| Location | Nucleotide exchange | dbSNP | Amino acid change | Domain | Proband | Proband’s father | Proband’s brother | Proband’s husband | Fetal |
|----------|--------------------|-------|------------------|--------|---------|-----------------|------------------|-----------------|-------|
| Exon 4   | c.523C > T         |       | Arg174X          | β-sandwich hetere              | hete          | hete             | hete             | hete             | hete  |
| Exon 10  | c.103G > T         | rs5985 | Val34Leu         | β-barrel-1                    | hete          | hete             | hete             | hete             | hete  |
| Intron 10| c.1149 - 34T       |       | Arg174pro        | Catalytic domain              | hete          | hete             | hete             | hete             | hete  |
| Exon 12  | c.1694C > T        | rs5982 | Pro564 Leu       | β-barrel-1                    | hete          | hete             | hete             | hete             | hete  |
| Intron 12| c.1769-34T         |       | Pro408Pro        | Catalytic domain              | hete          | hete             | hete             | hete             | hete  |
| Exon 14  | c.1951G > A        | rs5987 | Val650Ile        | β-barrel-2                    | hete          | hete             | hete             | hete             | hete  |
| Intron 14| c.1954G > C        | rs5988 | Glu651Gln        | β-barrel-2                    | hete          | hete             | hete             | hete             | hete  |
| Exon 14  | c.1954G > C        | rs5988 | Val650Ile        | β-barrel-2                    | hete          | hete             | hete             | hete             | hete  |
| Intron 14| c.2045 +39G > A    |       | Pro408Pro        | Catalytic domain              | hete          | hete             | hete             | hete             | hete  |
| 3’UTR    | c.*143G > A        | rs698859 | Sushi domains    | hete          | hete             | hete             | hete             | hete             | hete  |
| Exon 11  | c.1806G > C        |       | synonymous mutation | Sushi domains | hete          | hete             | hete             | hete             | hete  |

Notes: Except for the last two lines at the end of the table, which show the variations of the F13B gene, all the variations are detected from the F13A gene. hete, heterozygous mutation; hete, heterozygous; NI, not informative; N, normal and without mutations.
interactions. The highly charged Arg408 is localized in the immediate vicinity of the catalytic triad (Cys314–His373–Asp396), specifically Asp396. It changes its nature of side chains from the basic to rigid non-polar hydrophobic, then the substitution may probably induce a conformational change around the catalytic triad, which in turn would grossly damage the activity of the protein. Overall structure of Factor XIII-A2 dimers, a close-up view of the Arg408 and hydrogen bonds involving it is shown (Figure 5). We considered the novel mutation as a pathogenic mutation.

It is indicated that the deficiency of FXIII in the proband might be caused by the compound heterozygous mutations of the Arg174X nonsense mutation in exon 4 which was inherited through the maternal line and Arg408Pro missense mutation in exon 10 which was inherited through the paternal line. We successfully performed the PND for the pedigree by the procedures we have proposed and the child evaluated to be unaffected was successfully born in the month without bleeding complications, and the follow-up study verified our results.

Figure 3. Representative chromatograms from Sanger sequencing of the F13A gene in the pedigree. (I) Sequencing of c.523C > T (p.Arg174X) in the F13A gene, (a) proband, (b) proband’s father, (c) proband’s mother, (d) proband’s brother and (e) fetus. (II)Sequencing of 1226G > C (p.Arg408Pro) in the F13A gene, (a) proband, (b) proband’s father, (c) proband’s mother, (d) proband’s brother and (e) fetus.
Discussion

Among all the coagulation factor deficiencies, only FXIIID and FIB deficiencies were associated with pregnancy loss [28]. Pregnancy and delivery are challenging due to the increased risk of miscarriage, or PPH in pregnant woman with FXIIID and bleeding complications in a fetus with FXIIID, especially the CNS bleeding which is a major cause of death compared to other rare bleeding disorders [29,30]. While the risks can be minimized by PND which can facilitate the management or advance preparation for complications, it also provides suggestions for the mode of delivery which is a crucial factor in congenital bleeding disorders [31]. However, it does not indicate that normal vaginal delivery is absolutely contraindicated, and cesarean section may not completely eliminate the risk, but prolonged labor should be avoided and early recourse to cesarean section should be considered to minimize the risk.

When necessary, FXIII addition to the pregnant woman with FXIIID should be done before cesarean delivery.

The molecular method plays an essential role during the diagnosis, because it cannot be detected by routine coagulation tests, and some more specific tests still cannot be widely used due to their high costs, time-consumption and low sensitivity. In addition, the importance of determining the disease-causing mutation before embarking upon PND should be emphasized. Thus, performing timely PND with pathogenicity analysis for molecular results is the mainstay for prevention of life-threatening bleeding episodes in newborns and affected pregnant women.

PND is based on molecular techniques to analyze the fetal samples collected by chorionic villus sampling, amniocentesis, cordocentesis or free fetal DNA in maternal plasma, so benefits and limitations of each molecular technique should be known. For example, PCR-RFLP is a less cost-consuming and simple technique to screen mutations and amplification refractory mutation system is also applied for detecting mutations which are especially suitable for rapid processing of large numbers of samples, but both of them can only be used to detect the known mutations. Application of genetic sequencing technology in PND is more reliable. A combination of the next-generation sequencing with noninvasive PND technology is used as a more rapid and safer method recently. In our study, Sanger sequencing technique was used and its limitations must be considered, such as the occurrence of false-negative sequencing results from allele dropout [32], its inability of copy number variations detection and the drawbacks when encountered with chimeric sequences. And PCR also introduces artifacts into sequencing due to PCR bias or extreme GC composition of genomes. However, much attention needs to be paid to the specificity of the F13A gene; its polymorphism, which is not expected to be causative of disease, probably has association with a clinical presentation; it requires a rigorous interpretation with caution for polymorphisms. For instance, c.614A > T and c.1694C > T polymorphisms of the F13A gene are reported to be associated with RPL [33], and further genetic data need to be gathered for the validation; it also highlights the importance for case reports of FXIIID.

Undoubtedly, the report of novel mutations among populations will contribute to genetic diagnostics in certain districts due to the ethnic heterogeneity. We
provided a systematic procedure for PND with pathogenicity analysis of the novel mutation; it can not only enable a precise PND in limited time, but also lay the foundation for preimplantation molecular diagnosis and presymptom diagnosis for FXIIID, and contributes to the further study on genotype–phenotype correlation among the Chinese population. The comprehensive process for pathogenicity analysis will also facilitate the elimination of ambiguous interpretation of variants in clinics and improvement of the prospects for gene-based diagnostics.

**Conclusion**

In conclusion, to our knowledge this is the first study to establish a rapid and precise PND with pathogenicity analysis based on ACMG guidelines to facilitate timely management and eliminate life-threatening bleeding episodes. We also reported a novel pathogenic mutation to enrich the F13A gene mutations’ spectrum and contributed to genotype–phenotype correlation studies among the Chinese population.

**Acknowledgements**

We thank the patients and their families and healthy volunteers for participating in this study.

**Disclosure statement**

No potential conflict of interest was reported by the authors.

**Funding**

This study is supported by the Science and Technology Plan Project Grant of Guangdong Province (No. 2014A020213020, Recipient Weiyong Jiang; and Science and Technology Program Project of Guangzhou (No. 201604020020, Recipient Weiyong Jiang).

**Figure 5.** Structure prediction results. (A) Overall structure of Factor XIII-A2 dimers. Structure of FXIII-A2 is colored and labeled according to five domains (red, AP-FXIII; orange, β-sandwich domain; yellow, catalytic core region; green, β-barrel 1; blue, β-barrel 2), the red rectangle indicating the area between two homodimers which was magnified in figure C. (B) Surface model of FXIII-A2; the interface region between homodimers is highlighted in red and light green. The surface-exposed residues in the corresponding structure are colored and the buried residues are show in gray. (C) Magnified area between two homodimers to show the salt bridge between Arg260 amino acid in one FXIIIA monomer and Asp404 of another monomer which is crucial in the FXIII-A2 homodimer formation. (D) Close-up view of the position of Arg408. Hydrogen bonds involving the Arg408 are shown as strings of small red spheres indicates Arg408 amino acid interacts with Arg260 amino acid by hydrogen bounding.
Notes on contributors

Liwei Sun is a Master’s candidate in Sun Yat-sen University of Medical Genetics.
Qi Jiang Yan is a researcher at Guangzhou Kingmed Diagnostics Technology Co., LTD.
Bin Wan is a faculty member at The First Affiliated Hospital of Guangzhou Medical University.
Yonghua Wang is a Master’s candidate in Sun Yat-sen University of Medical Genetics.
Hualei Luo is a Master’s candidate in Sun Yat-sen University of Medical Genetics.
Peng Du is a Master’s candidate in Sun Yat-sen University of Medical Genetics.
Reem Hassan is a Ph.D. candidate in Sun Yat-sen University of Medical Genetics.
Li Liu is a professor at the department of genetics and endocrinology, Guangzhou Women and Children’s Medical Center.
Weiying Jiang is a professor in ZhongShan School of Medicine, Sun Yat-sen University. She is an expert in the field of Medical Genetics.

References

[1] Dorgalaleh A, Rashidpanah J. Blood coagulation factor XIII and factor XIII deficiency. Blood Rev. 2016;30 (6):461–475. DOI:10.1016/j.blre.2016.06.002. PubMed PMID: 27344554; eng.
[2] Biswas A, Ivaskevicius V, Seitz R, et al. An update of the mutation profile of factor 13 A and B genes. Blood Rev. 2011;25(3):193–204. DOI:10.1016/j.blre.2011.03.001. PubMed PMID: 21640452; eng.
[3] Bagoly Z, Koczi Z, Haraszlavi J, et al. Factor XIII, clot structure, thrombosis. Thromb Res. 2012;129(3):382–387. DOI:10.1016/j.thromres.2011.11.040. PubMed PMID: 22197181; eng.
[4] Ichinose A, Davie EW. Characterization of the gene for the a subunit of human factor XIII (plasma transglutaminase), a blood coagulation factor. Proc Natl Acad Sci U S A. 1988;85(16):5829–5833. PubMed PMID: 2901091; PubMed Central PMCID: PMC281858. eng.
[5] Duan B, Wang X, Chu H, et al. Deficiency of factor XIII gene in Chinese: 3 novel mutations. Int J Hematol. 2003;78(3):251–255. PubMed PMID: 14604285; eng.
[6] Zheng WD, Liu YH, He QY, et al. Identification of Arg77Cys and Arg174stop double heterozygous mutation in a Chinese family with inherited FXIII deficiency. Zhonghua Xue Ye Xue Za Zhi. 2009;30 (3):158–161. PubMed PMID: 19642362; chi.
[7] Wu S, Wang Z, Dong N, et al. A novel compound heterozygous mutation in the F13A gene causing hereditary factor XIII deficiency in a Chinese family. J Thromb Haemost. 2006;4(1):267–269. DOI:10.1111/j.1538-7836.2005.01699.x. PubMed PMID: 16409483; eng.
[8] Xu G, Liang Q, Zhang L, et al. Identification of genetic defects in a Chinese pedigree with factor XIII deficiency: case report and literature review. Zhonghua Xue Ye Xue Za Zhi. 2015;36(10):844–848. DOI:10.3760/cma.j.issn.0253-2727.2015.10.008. PubMed PMID: 26477763; chi.
[9] Wang W, Huang L, Ma Q, et al. Homozygous intronic mutation leading to inefficient transcription combined with a novel frameshift mutation in F13A1 gene causes FXIII deficiency. J Hum Genet. 2011;56(6):460–463. DOI:10.1038/jhg.2011.41. PubMed PMID: 21512576; eng.
[10] Jiao WY, Wu JS, Ding QL, et al. Identification of a novel mutation of F (13) A gene in a pedigree with factor XIII deficiency. Zhonghua Xue Ye Xue Za Zhi. 2007;28 (9):598–601. PubMed PMID: 18246815; chi.
[11] Naderi M, Alizadeh S, Kazemi A, et al. Central nervous system bleeding in pediatric patients with factor XIII deficiency: a study on 23 new cases. Hematology. 2015;20(2):112–118. DOI:10.1179/1607845414y.0000000172. PubMed PMID: 25001244; eng.
[12] Behboudi-Gandevani S, Moghadam-Banaei L, Shahbazi S, et al. Maternal rare inherited bleeding disorders and neonatal complications. J Obstet Gynaecol Res. 2016;42(2):172–177. DOI:10.1111/jog.12884. PubMed PMID: 26627666; eng.
[13] Daffos F, Foretisler F, Kaplan C, et al. Prenatal diagnosis and management of bleeding disorders with fetal blood sampling. Am J Obstet Gynecol. 1988;158 (4):939–946. PubMed PMID: 3364504; eng.
[14] Shanbhag S, Ghosh K, Shetty S. First trimester prenatal diagnosis of severe FXIII deficiency. Haemophilia. 2016;22(5):e443–e444. DOI:10.1111/hae.12982. PubMed PMID: 27340806; eng.
[15] Kangsadalampai S, Coggan M, Caglayan SH, et al. Application of HUMF13A01 (AAAG)n STR polymorphism to the genetic diagnosis of coagulation factor XIII deficiency. Thromb Haemost. 1996;76(6):879–882. PubMed PMID: 8972004; eng.
[16] Killick CJ, Barton CJ, Aslam S, et al. Prenatal diagnosis in factor XIII-A deficiency. Arch Dis Child Fetal Neonatal Ed. 1999;80(3):F238–F239. PubMed PMID: 10212091; PubMed Central PMCID: PMC30172090. eng.
[17] Naderi M, Reykande SE, Dorgalaleh A, et al. Establishment of a prenatal diagnosis schedule as part of a prophylaxis program of factor XIII deficiency in the southeast of Iran. Blood Coagul Fibrinolysis. 2016;27(1):97–100. DOI:10.1097/mbc.0000000000000374. PubMed PMID: 26703985; eng.
[18] Jennings I, Kitchen S, Woods TA, et al. Problems relating to the laboratory diagnosis of factor XIII deficiency: a UK NEQAS study. J Thromb Haemost. 2003;1(12):2603–2608. PubMed PMID: 14675096; eng.
[19] Dorgalaleh A, Tabibian S, Hosseini MS, et al. Diagnosis of factor XIII deficiency. Haematologica. 2016;21(7):430–439. DOI:10.1080/10245332.2015.1101975. PubMed PMID: 27077776; eng.
[20] Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405–424. DOI:10.1038/gim.2015.30. PubMed PMID: 25741868; PubMed Central PMCID: PMC4454735. eng.
[21] Sharief LA, Kadir RA. Congenital factor XIII deficiency in women: a systematic review of literature. Haemophilia. 2013;19(6):e349–e357. DOI:10.1111/hae.12259. PubMed PMID: 23992439; eng.
[22] Reiner AP, Frank MB, Schwartz SM, et al. Coagulation factor XIII polymorphisms and the risk of myocardial infarction and ischaemic stroke in young women. Br J Haematol. 2002;116(2):376–382. PubMed PMID: 11841441; eng.
[23] Anwar R, Miloszewski KJ, Markham AF. Identification of a large deletion, spanning exons 4 to 11 of the human
factor XIII A gene, in a factor XIII-deficient family. Blood. 1998;91(1):149–153. PubMed PMID: 9414279; eng.

[24] Anwar R, Stewart AD, Miloszewski KJ, et al. Molecular basis of inherited factor XIII deficiency: identification of multiple mutations provides insights into protein function. Br J Haematol. 1995;91(3):728–735. PubMed PMID: 8555083; eng.

[25] Castaman G, Giacomelli SH, Ivaskevicius V, et al. Molecular characterization of five Italian families with inherited severe factor XIII deficiency. Haemophilia. 2008;14(1):96–102. DOI:10.1111/j.1365-2516.2007.01603.x. PubMed PMID: 18028394; eng.

[26] Louhichi N, Medhaffar M, HadjSalem I, et al. Congenital factor XIII deficiency caused by two mutations in eight Tunisian families: molecular confirmation of a founder effect. Ann Hematol. 2010;89(5):499–504. DOI:10.1007/s00277-009-0863-y.

[27] Muszbek L, Yee VC, Hevessy Z. Blood coagulation factor XIII. Thromb Res. 1999;94(5):271–305. PubMed PMID: 10379818; eng.

[28] Inbal A, Muszbek L. Coagulation factor deficiencies and pregnancy loss. Semin Thromb Hemost. 2003;29(2):171–174. DOI:10.1055/s-2003-38832. PubMed PMID: 12709920; eng.

[29] Dorgalaleh A, Tabibian S, Shams M, et al. Laboratory diagnosis of factor XIII deficiency in developing countries: an Iranian experience. Lab Med. 2016;47(3):220–226. DOI:10.1093/labmed/lmw021. PubMed PMID: 27346867; PubMed Central PMCID: PMCPmc4985770. eng.

[30] Dorgalaleh A, Naderi M, Hosseini MS, et al. Factor XIII deficiency in Iran: a comprehensive review of the literature. Semin Thromb Hemost. 2015;41(3):323–329. DOI:10.1055/s-0034-1395350. PubMed PMID: 25615432; eng.

[31] Naderi M, Eshghi P, Cohan N, et al. Successful delivery in patients with FXIII deficiency receiving prophylaxis: report of 17 cases in Iran. Haemophilia. 2012;18(5):773–776. DOI:10.1111/j.1365-2516.2012.02785.x. PubMed PMID: 22458944; eng.

[32] Tsai AC, Hung YW, Harding C, et al. Next generation deep sequencing corrects diagnostic pitfalls of traditional molecular approach in a patient with prenatal onset of Pompe disease. Am J Med Genet A. 2017;173(9):2500–2504. DOI:10.1002/ajmg.a.38333. PubMed PMID: 28657663; eng.

[33] Jeddi-Tehrani M, Torabi R, Mohammadzadeh A, et al. Investigating association of three polymorphisms of coagulation factor XIII and recurrent pregnancy loss. Am J Reprod Immunol. 2010;64(3):212–217. DOI:10.1111/j.1600-0897.2010.00838.x. PubMed PMID: 20384622; eng.