

Original Article

Thrombin Activatable Fibrinolysis inhibitor Thr 325 Ile polymorphism in fetuses with factor XIII deficient family history and Intracranial hemorrhage

Majid Naderi¹, Samira Esmaili-Reykande^{2,3}, Shaban Alizadeh^{2*}, Akbar Dorgalaleh², Zahra Sadat Abtahi², Zahra Kashani-Khatib⁴, Saied Kaviani⁴

¹ Genetic Researcher Center in Non-Communicable Disease, Zahedan University of Medical sciences, Zahedan, Iran

² Department of Hematology, Allied Medical School, Tehran University of Medical Sciences, Tehran, Iran

³ Student Scientific Research Center, School of Allied Medicine, Tehran University of Medical Sciences, Tehran, Iran

⁴ Department of Hematology, Faculty of Medical sciences, Tarbiat Modares University, Tehran, Iran

Received: 29 October, 2015; Accepted: 6 January , 2016

Abstract

Background: Factor XIII Deficiency (FXIID) is an inherited rare bleeding disorder with some life threatening clinical manifestation including Intracranial Haemorrhage (ICH). Among all polymorphisms found in FXIID, Thrombin Activatable Fibrinolysis Inhibitor (TAFI) Thr325Ile gene polymorphism increases probability of ICH about 20 fold in patients with FXIII .So, in this study we aimed to evaluate TAFI Thr 325 Ile polymorphism in Chorionic villus samples (CVS) of fetuses with positive family history of FXIID and ICH. **Materials and Methods:** This study was performed on chorionic villus of pregnant mothers with positive history of FXIID accompanied with ICH in first-degree relatives of their fetus. All parents of the fetuses were completed consent form for doing Prenatal diagnosis (PND). Chorionic villus DNA was extracted from each sample using the DNA extraction kit and PCR-RFLP was performed for TAFI Thr 325Ile polymorphism in Exon 4 of FXIII A gene. **Results:** All of 8 fetuses had positive family history of FXIID. Seven out of eight fetuses (87.5%) had a family member with CNS bleeding due to FXIID. Four fetuses had history of death due to FXIID. There were 5 case (62.5%) that were homozygote for TAFI Thr 325 Ile, one (12.5%) was heterozygote and two (25%) were non mutant. **Conclusion:** Detection of TAFI Thr 325 Ile polymorphism by PND program in fetuses with positive family history of ICH is seems necessary and it will help to fill many gaps in preventing life threatening features of FXIID in newborn at the time of delivery by prophylaxy receiving and precautionary measures.

Keywords: Factor XIII deficiency, intracranial hemorrhage, TAFI Thr 325Ile

*Corresponding Author: Shaban Alizadeh, Department of Hematology, Allied Medical School, Tehran University of Medical Sciences, Tehran, Iran. E-mail: alizadehs@sina.tums.ac.ir.

Please cite this article as: Naderi M, Esmaili-Reykande S, Alizadeh Sh, Dorgalaleh A, Abtahi ZS, Kashani-Khatib Z, Kaviani S. Thrombin Activatable Fibrinolysis inhibitor Thr 325 Ile polymorphism in fetuses with factor XIII deficient family history and Intracranial hemorrhage. Arch Med Lab Sci. 2015;1(3): 102-6.

Introduction

Factor XIII deficiency (FXIID) is an inherited rare bleeding disorder (RBD) that was presented with different clinical manifestation such as life-long bleeding tendency, recurrent miscarriages,

spontaneous abortion in affected women, mucosal bleeding and life-threatening Intracranial Hemorrhage (ICH). ICH is an irreparable outcomes of severe FXIID, and an early diagnosis of the disease followed by a long-term prophylactic treatment is crucial (1-4). ICH is an important cause of morbidity and mortality

in infancy affected with FXIID. ICH may occurred in the neonatal, perinatal and infant periods (5). Cerebral hemorrhage is a kind of common event that its relevance to the most genetic disorders such as thrombin activable fibrinolysis inhibitor (TAFI) was evaluated. TAFI is a procarboxypeptidase that stabilizes fibrin clots, and some mutations in this molecule cause increase in plasma level of it to 60% that can cause thrombotic complications such as myocardial infraction and stroke. Thr 325 Ile is one of the most common polymorphism of TAFI gene that its relevance of stroke was approved (6, 7). In our last study, we concluded that TAFI Thr325Ile gene polymorphism increases probability of ICH in patients with positive FXIII Trp 187 Arg mutation about 20 fold in comparison with control group (2). This mutation is the most common mutation in FXIID in Iranian patients (8). Since the first degree relative families of fetuses had approved FXIII Trp 187 Arg accompanied with CNS bleeding, thus in this study we aimed to evaluate TAFI Thr 325 Ile polymorphism in chorionic villus samples of fetuses to estimate CNS bleeding and prevent of this event in affected fetuses during birth and after that with long-term prophylactic treatment.

Methods

This descriptive study was carried out on families with positive history of FXIID and CNS bleeding. FXIII Trp187Arg mutation (C.559T>C) as a mutation related to almost all patients with FXIID in south east of Iran was selected for determination of FXIID for these family members and fetus status. Then, families with positive history of FXIID were candidate to undergo to perform prenatal diagnosis (PND) of FXIID. chorionic villus samples (CVS) of eight pregnant women in their first trimester who referred to hemophilia center in Sistan and

Baluchestan Province in southeast of Iran during September 2013 to March 2014. All of eight fetuses had positive history of FXIID in their first degree relatives. All parents of the fetuses were completed consent form. This study was approved by the medical ethics committee of Tehran University of Medical Sciences.

PND process was done by radiologist and chorionic villus sample (CVS) was given from the amniotic cavity of uterus and suitable amount of vilii was extracted.

Chorionic villus DNA was extracted from each sample with the DNA extraction kit (Viogene). The quality of obtained DNA was determined by means of 2% agarose gel electrophoresis.

FXIII TAFI325Ile Thr polymorphism was amplified under standard conditions as shown on table 1.

The PCR product was digested with SpeI (BcuI) (Fermentase Life Sciences, York, UK) according to the manufacturer's instruction. Finally the digested products were resolved on a 2% agarose gel containing 0.5 µg/ml ethidium bromide.

Results

All of 8 fetuses had positive family history with confirmed FXIID with Trp 187 Arg. Seven out of eight fetuses (87.5%) had a family member with CNS bleeding due to FXIID. Four fetuses(50%) had a positive history of death due to FXIID. Umbilical cord bleeding and ecchymosis were positive in all members of fetus families. Other demographic and clinical manifestations of members of fetus families were given in table 2.

As shown in figure1, in fetus samples, we had 5 cases (62.5%) as the homozygote, one case (12.5%) as the heterozygote and two cases(25%) as the non mutant.

The first fetus was heterozygous of Trp 187

Table 1: Primers and PCR condition for Trp187Arg mutation.

Primer sequences	Number of Cycles	Annealing temperature	PCR Product (bp)	Fragments size after digestion (bp)
Forward :5'-TGC TTC CAG TCCTA GTA GC-3'	30	56°	216bp	WT:216 Hetero: 166, 50 Homo:216, 166, 50 bp fragments
Reverse: 5'-CAG TTG TAT TACATG TGA CC-3'				

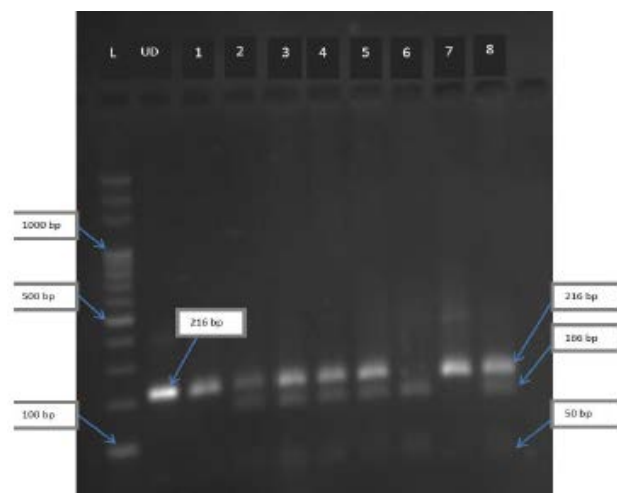


Fig. 1. TAFI polymorphism Thr 325 Ile of 8 fetuses. L:Ladder 100bp, UD: undigested product, 1,7: unmutant, 2,3,4,5,8: homozygote ,6: heterozygote

Table 2: Demographic characteristics and clinical manifestations of familial history of fetuses.

Case	Gum bleeding	History of death due to FXIID	History of CNS bleeding
1	Pos	Pos	Pos
2	Pos	Neg	Pos
3	Pos	Neg	Neg
4	Neg	Pos	Pos
5	Neg	Neg	Pos
6	Neg	Pos	Pos
7	Neg	Neg	Pos
8	Neg	Pos	Pos

Pos: positive, Neg: Negative

Arg mutation and no polymorphism in TAFI 325 Ile gene . His/Her parents were heterozygous for these mutations. The family had two children with severe FXIID and also had positive history of death related to FXIID.

Fetus 2 was homozygous of Trp 187 Arg and TAFI 325 Ile polymorphism, and his/her parents are first degree relatives . This family has a daughter with FXIID clinical manifestations such as bleeding from the umbilical cord, ecchymosis, gum bleeding and CNS bleeding . The family had no death history related to FXIID.

The third fetus was without Trp 187 Arg mutation and homozygous of TAFI 325 Ile gene polymorphism. His/Her parents were heterozygous mutation and were first degree relative.

The fourth embryonic case had no Trp 187 Arg mutation and homozygous polymorphism of

TAFI Thr 325 Ile . Her/His parents were heterozygous for this mutation, and they were first degree relatives.

The fifth fetus was from parents with first degree relative family and heterozygous for Trp 187 Arg mutation . The fetus had no this mutation and homozygous polymorphisms of TAFI 325 Ile gene. This family had death related to FXIID and also had clinical manifestation related to FXIID include umbilical cord bleeding, ecchymosis , gum bleeding and CNS bleeding.

Sixth fetus was heterozygous of Trp 187 Arg mutation and heterozygous polymorphisms of TAFI 325 Ile .His/Her parents were not family relatives, but the number of affected members of this family with FXIID was two. Molecular study was showed that the mother was homozygous of Trp 187 Arg mutation and father was heterozygous. The most important clinical manifestations related to FXIID were umbilical cord blood, ecchymosis and gum bleeding in this family. The history of CNS bleeding and death related to FXIID in this family were negative.

The Seventh fetus was heterozygous of Trp 187 Arg mutation with no polymorphism in TAFI 325 Ile gene. His/Her parents were heterozygous of Trp 187 Arg mutation. This family has a daughter with FXIID with umbilical cord bleeding, ecchymosis, gum bleeding and CNS bleeding. The family had no death related to FXIID .

Eighth embryonic sample revealed no mutation of Trp 187 Arg mutation and homozygous TAFI 325 Ile polymorphism .His/Her parents were heterozygous for this mutation. The family has three children with a severe FXIID and the most clinical manifestations of the three children were umbilical cord blood, ecchymosis and CNS bleeding. The family had two deaths related to FXIID.

Discussion

Sistan and Baluchestan Province in southeastern of Iran with 352 patients with FXIID, has the highest incidence of the disorder not only in Iran but also in all over the world because of high consanguineous marriages (8,10). These patients cause a high rate of morbidity and mortality among Iranian patients and CNS bleeding is the main cause of death among this population (11-14). Umbilical cord bleeding is the

most common clinical manifestation pattern of FXIID in Iranian patients (4). The most important clinical manifestation of FXIID in our study is CNS and umbilical cord blood bleeding, ecchymosis and gum bleeding.

TAFI ,1040CT(Thr/Ile) is in the coding region of the TAFI gene and this polymorphism affects both plasma antigen level , its activity and stability of TAFI in *in vitro* .The CC (Thr/Thr) genotype was associated with the highest levels of TAFI and the TT (Ile/Ile) genotype was associated with the lowest ones. Ile-325 variants exhibited an antifibrinolysis effect that was 60% greater than that of Thr-325 variants (8).

In our study, we had 5 cases with homozygote of TAFI Thr 325 Ile polymorphism .We concluded that TAFI Thr325 Ile polymorphism is an important prognostic factor of CNS bleeding in patients with FXIID and increases the risk of CNS bleeding by 20 fold .The bleeding polymorphism of TAFI Thr 325 Ile in accompanied with Trp 187 Arg gene mutation of FXIII can increase the risk of bleeding in CNS, so identification of homozygous of TAFI Thr 325 Ile polymorphism in fetuses recommend more importance of birth care of the fetus during birth and after that , fetus had this characteristics (2). Tokgoz *et al*, reported that there was no correlation between this polymorphism and stroke but Kozian *et al* reported a clear correlation between TAFI C>T1040 and stroke (16, 17).

In spite of high prevalence of hemophilia, there is no evidence-based guidelines for the management of delivery in carriers of hemophilia pregnant women . There is different options for the delivery of these women that it seems recommended vaginal delivery, and cesarean doesn't eliminate the risk of intracranial hemorrhage in newborns with hemophilia . Most studies are about hemophilia and cesarean delivery has less justification than normal delivery, but due to higher frequency of CNS bleeding in patients with FXIID, caesarean is more suitable (18-22).

Affected women at reproductive age are usually complicated by menorrhagia and they are highly prone to recurrent miscarriage at their pregnancy period (23). So, it is recommended that they receive 10IU/Kg Fibrogammin concentrate

during their pregnancy every two weeks and have cesarean delivery.

In our study, fetus 2 was homozygous of Trp 187 Arg and TAFI 325 Ile polymorphism. So, his/her susceptibility to bleeding in bleeding polymorphism of TAFI 325 Ile is high. Since the mother is homozygous for the Trp 187 Arg mutation, it is recommended that she receive 10IU/Kg Fibrogammin concentrate during her pregnancy every two weeks and have cesarean delivery. Also, the baby receive Fibrogammin concentrate soon after birth to prevent CNS bleeding and other life-threatening bleeding.

Sixth fetus was heterozygous of Trp 187 Arg mutation and heterozygous polymorphisms of TAFI 325 Ile . Molecular study was showed that the mother was homozygous of Trp 187 Arg mutation and father was heterozygous. We recommend that the mother receive Fibrogammin concentrate 10 IU/Kg during her pregnancy every two weeks and she have cesarean delivery to prevent life-threatening bleeding. Security measures for the fetus should be done during and after birth.

Our limitation was low related study and there was just one study about PND of FXIID in the literature (24) that we couldn't discuss and compare more about this subject. We suggest that PND of Trp 187 Arg mutation of FXIII gene be established with TAFI Thr 325 Ile in families in the regions with high frequency of FXIID and CNS bleeding.

Conclusion

ICH is a life threatening complication of FXIID and it is commonly occur in these patients. Thus, detection of TAFI polymorphism by PND program in fetuses with positive family history of ICH is necessary and it will fill many gaps in preventing life threatening features of FXIID in newborn at the time of delivery by receiving prophylaxy and precautionary measures.

Conflicts of Interest

The authors declare that there is no conflict of interest in this study.

Acknowledgment

This research has been supported by Tehran university of Medical Sciences and health services grant 25286.

References

- Naderi M, Dorgalaleh A, Alizadeh S, Kashani Khatib Z, Tabibian S, Kazemi A, Dargahi H, Bamedi T. Polymorphism of thrombin-activatable fibrinolysis inhibitor and risk of intracranial haemorrhage in factor XIII deficiency. *Haemophilia*. 2014;20(1):e89-e92.
- Naderi M, Dorgalaleh A, Alizadeh SH, Kazemi A, Dargahi H, Younedi MR, et al. Assessment of relationship between CNS bleeding in factor XIII deficiency and Thrombin- Activatable Fibrinolysis Inhibitor polymorphism. *Arak Medical University Journal*. 2013; 16(7): 83-90.
- Naderi M, Dorgalaleh A, Alizadeh S, Kazemi A, Tabibian S, et al. Central Nervous System Bleeding in Factor XIII Deficiency, Assessment the Role of Plasminogen Activator Inhibitor-1. *Journal of Isfahan Medical School*. 2014; 31(263): 1985-1993.
- Naderi M, Dorgalaleh A, Tabibian S, Alizadeh S, Eshghi P, Solaimani G. Current understanding in diagnosis and management of factor XIII deficiency. *Iran J Pediatr Hematol Oncol*. 2013; 3(4): 164-72.
- Smith C. Intracranial haemorrhage in infants. *Current Diagnostic Pathology*, 2006. 12(3): 184-190.
- Karimi M, Bereczky Z, Cohan N, Muszbek L. Factor XIII deficiency. *Semin in Thromb Hemost*. 2009, 35(4):426-438.
- Mosnier LO, Bouma BN. Regulation of fibrinolysis by thrombin activatable fibrinolysis inhibitor, an unstable carboxypeptidase B that unites the pathways of coagulation and fibrinolysis. *Arteriosclerosis, thrombosis, and vascular biology*, 2006. 26(11): 2445-2453.
- Dorgalaleh A, Naderi M, Hosseini MS, Alizadeh S, Hosseini S, Tabibian S. Factor XIII deficiency in Iran: a comprehensive review of the literature. *Semin Thromb Hemost*. 2015;41(3):323-9.
- Naderi M, Esmaeili Reykande S, Dorgalaleh A, Alizadeh S, Einolahi N, Tabibian S, et al. Establishment of a prenatal diagnosis schedule as part of prophylaxis program of factor XIII deficiency in southeast of Iran. *Blood coagulation and fibrinolysis*. 2015.(in press)
- Naderi M, Imani M, Eshghi P, Dorgalaleh A, Tabibian S, Alizadeh S, Sanei Moghadam E, Miri moghadam E. Factor XIII deficiency in Sistan and Baluchistan province. *Sci J Blood Transfus Organ*. 2013; 10(3): 282-288.
- Naderi M, Alizadeh S, Kazemi A, Tabibian S, Zaker F, Bamedi T, Kashani Khatib Z, Dorgalaleh A. Central nervous system bleeding in pediatric patients with factor XIII deficiency: A study on 23 new cases. *Hematology*. 2015.20(2):112-8.
- Majid N, Younesi MR, Dorgalaleh A, Alizadeh S, Kazemi A, Tabibian S. Association between expression of MMP-2 and MMP-9 genes and pathogenesis of intracranial hemorrhage in severe coagulation factor XIII deficiency. *Hematology*. 2015.(in press)
- Naderi M, Zarei T, Haghpanah S, Eshghi P, Miri-Moghaddam E, Karimi M. Intracranial hemorrhage pattern in the patients with factor XIII deficiency. *Annals of hematology*. 2014;93(4):693-7.
- Peyvandi F, Tagliabue L, Menegatti M, Karimi M, Komáromi I, Katona É, Muszbek L, Mannuccio Mannucci P. Phenotype- genotype characterization of 10 families with severe a subunit factor XIII deficiency. *Human mutation*. 2004;23(1):98-108.
- Akatsu H, Yamagata H, Chen Y, Miki T, Kamino K, Takeda M, et al. TAFI polymorphisms at amino acids 147 and 325 are not risk factors for cerebral infarction. *British journal of haematology*, 2004. 127(4): 440-447.
- Tokgoz S, Zamani AG, Durakbasi-Dursun HG, Yilmaz O, Ilhan N, et al., TAFI gene polymorphisms in patients with cerebral venous thrombosis. *Acta neurologica Belgica*, 2013. 113(3): 291-297.
- Kozian DH, Lorenz M, März W, Cousin E, Mace S, Deleuze J. Association between the Thr325Ile polymorphism of the thrombin-activatable fibrinolysis inhibitor and stroke in the Ludwigshafen Risk and Cardiovascular Health Study. *Thrombosis & Haemostasis*, 2010. 103(5): 976-983.
- Kulkarni R, Lusher JM, Henry RC, Kallen DJ. Current practices regarding newborn intracranial haemorrhage and obstetrical care and mode of delivery of pregnant haemophilia carriers: a survey of obstetricians, neonatologists and haematologists in the United States, on behalf of the National Hemophilia Foundation's Medical and Scientific Advisory Council. *Haemophilia* .1999; 5:410-415.
- Street AM, Ljung R, Lavery AM. Management of carriers and babies with haemophilia. *Haemophilia* .2008, 14 (3), 181-187.
- Yoffe G, Buchanan GR. Intracranial hemorrhage in newborn and young infants with haemophilia. *J Pediatr* 1998 ;113: 333-6.
- Ljung R, Lindgren AC, Petrini P et al. Normal vaginal delivery is to be recommended for haemophilia carrier gravidae. *Acta Paediatr*. 1994; 83: 6901-11.
- Goldsmith JC, Kletzel M. Risk of birth related intracranial hemorrhage in haemophilic newborns: Results of a North American survey . *Blood* .1990;76: 421.
- Levy JH, Greenberg C. Biology of Factor XIII and clinical manifestations of Factor XIII deficiency. *Transfusion (Paris)*. 2013;53(5):1120-31.
- Killick CJ, Barton CJ, Aslam S, Standen G. Prenatal diagnosis in factor XIII-deficiency. *Arch Dis Child Educ Pract Ed* 1999;80(3): 238-9.