Case Report

Identification of the First Iranian Family with "γArg275Cys" Mutation (Fibrinogen Tokyo II)

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Abstract

Background: Inherited fibrinogen deficiencies are classified into two categories: quantitative (including a fibrinogenemia and hypofibrinogenemia) and qualitative (including dysfibrinogenemia). Any mutation in fibrinogen genes accounts for one of these disorders.

Case Report: This article reports an Iranian family with dysfibrinogenemia without any clinical signs accidentally diagnosed by routine coagulation tests with slightly elevated PT and APTT a few years ago. For determination of the disease which causing genetic aberration in fibrinogen genes, DNA sequencing of three hot spots of these genes (i.e. exon 2 of FGA, exon 2 of FGB and exon 8 of FGG) was performed. Analysis of sequencing results revealed a heterozygous missense mutation c.901 C>T (Arg275Cys) in exon 8 of FGG in mother and children. No mutation was detected in father's sample. Fibrinogen with this mutation is known as Tokyo II.

Conclusion: γArg275Cys is a heterozygous mutation that impairs the function of fibrinogen and has been solely reported in dysfibrinogenemic patients. Clinical findings in this family (no history of bleeding and thrombosis) were compatible with molecular results, because fibrinogen Tokyo II does not have a thrombotic or hemorrhagic nature and lack of clinical signs in this family is not unexpected.

Keywords: Fibrinogen, Tokyo II, Dysfibrinogenemia

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Introduction

In hepatocytes, transcription of three clustered genes of FGA, FGB and FGG leads to production of three polypeptide chains Aα, Bβ, and γ, eventually causes fibrinogen formation. This factor is secreted to blood with normal plasma level of 1.5-3.5 gr/L (1). Inherited fibrinogen deficiencies are rare bleeding disorders (RBDs) that classified into two categories: quantitative, including a fibrinogenemia (factor level <0.1 gr/L) and hypofibrinogenemia (factor level 0.5- 1.5 gr/L) as well as qualitative, including dysfibrinogenemia that is characterized with abnormal function of fibrinogen (2). The main goal of coagulation cascade is conversion of fibrinogen to fibrin polymers and clot formation (1). Mutation in one of the fibrinogen genes can account for any of these disorders. The most important causative mutations of congenital dysfibrinogenemia (CD) have been identified in exon 2 of FGA, exon 2 of FGB and exon 8 of FGG (3). Here we described the first

dysfibrinogenemic Iranian family with γArg275Cys mutation bearing fibrinogen Tokyo II which was not reported previously in Kermanshah Province.

Case Presentation

This study was performed in 2015 and reports a family with consanguineous marriage from Kermanshah Province, Sahne city of Iran. Mother of case was diagnosed as dysfibrinogenemic just during routine coagulation tests before caesarian section 16 years ago. Prolonged prothrombin time (PT) and slightly elevated activated partial thromboplastin time (APTT) along, with abnormal fibrinogen activity measured by Clause method were indicative of dysfibrinogenemia. Moreover, lack of liver failure symptoms like increased plasma liver enzymes excluded acquired form of the disorder. Two sons were screened for disease when they were one year old and CD was confirmed in them. Table 1 displays the results of coagulation tests in this family. As it is evident in Table 1, the father was apparently healthy. Considering the clinical manifestations, none of the members of this family had experienced bleeding (such as menorrhagia and postpartum hemorrhage in mother, epistaxis, oral cavity bleeding, bleeding after dental extraction and hematomas) or thrombosis.

This study was continued by molecular tests for determination of disease causing genetic aberration in fibrinogen genes. After receiving the consent form and sampling, DNA was extracted from

Table 1: Coagulation test results in all the family members.

Members	PT(sec)	APTT (sec)	FC*(functional assay) (gr/L)
Father	12	30.5	2.6
Mother	17	38	0.45
Son 1	17.6	38.3	0.47
Son 2	16.7	40.6	0.6

Prothrombin Time/Normal range: 11.8 -14.6 second
Activated Partial Thromboplastin Time/Normal range: 30-38 second. * Fibrinogen concentration.Normal range: 1.5-3.5 gr/L

whole blood of all family members using DNA extraction kit (Blood & Tissue Genomic DNA Extraction Miniprep System; Viogene, Taipei, Taiwan). After that, DNA was used for amplification of hot spots CD variation according to updated fibrinogen mutation GEHT web site (www.geht.org/databaseang/fibrinogen), including exon 2 of FGA, exon 2 of FGB and exon 8 of FGG. The primers used for PCR amplification and sequencing of these three exons as well as PCR information are shown in Table 2. After purification of PCR products by agarose gel extraction, they were cycle sequenced by forward and reverse primers.

Interpretation of cycle sequencing results by Mutation Surveyor version 3.3 and Chromas version 2.4.3 software revealed a heterozygous substitution of C>T at position 901 of cDNA (c.901 C>T) in exon 8 of FGG, which leads to replacement of arginine by cysteine at position 275 of mature gamma chain (position 301 in immature form). This missense mutation is a known disease causing variant "rs12913087" for CD. The fibrinogen bearing this mutation is named *Tokyo II* (Other names: Osaka 2 /Tochigi 1 /Moricka 1). As expected, this variation was not found in the sample taken from healthy father as expected (Figure 1).

Discussion

We have reported the first Iranian family with fibrinogen Tokyo II. Despite the high prevalence of fibrinogen disorders in Iran in comparison to other countries, molecular investigation of this disorder is uncommon in this province.

According to World Federation of Hemophilia (WFH) in 2013, Iran has highest number of patients with inherited fibrinogen disorders (5). Because of the presence of some cases with asymptomatic dysfibrinogenemia (like this family), a larger number of patients is estimated in communities.

Arg275Cys mutation involves a C-terminal region of gamma chain, which is crucial for end to end alignment at fibrin monomers. So, this amino acid exchange is thought to be associated with abnormal interaction in fibrin monomers (6).

In comparison to known thrombotic mutation Arg573Cys in alpha chain of fibrinogen in CD patients, Arg275Cys in gamma chain does not involve

Table 2: Primers used for amplification and sequencing.

| Fibrinogen gene	Forward	Reverse	Annealing temperature (°C)	Amplicon size (bp)
FGA exon 2	TGAGAGTGCCATCTC TTCCTG	AAATCCTGTCTGTTCACC CACT (4)	58	440
FGB exon 2	GAGGGTGTTGGAATA GTTACA	ACAGGCTTTCTCTGCATG AG	53	320
FGG exon 8	TTCCAAGGAAGCATC CTAC	GTCTAAAGGAGATCCCA CAAC	55.5	657

thrombotic and bleeding effects (7).

Two mutations of Arg275Cys and Arg275His were identified in 17% of dysfibrinogenemic patients from UK (8). Also, more comprehensive study at Finland on 101 affected subjects reported that Arg275Cys mutation is the most common cause of dysfibrinogenemia with prevalence of 32.7% (9). Nevertheless, another study has indicated Arg16Cys mutation in alpha chain as the main variation of hot spot in dysfibrinogenemia (10). So, mutations detected incase reports or case series with dysfibrinogenemia can provide more data for resolution of these conflicts.

The presence of two affected sons in a family with healthy father and carrier mother (heterozygous mutation) may be justified by autosomal dominant inheritance pattern of CD. So far, almost all of the mutations detected in CD subjects have been heterozygous.

Conclusion

Arg275Cys is a heterozygous mutation that impairs the function of fibrinogen and has been solely reported in dysfibrinogenemic patients. Clinical findings in this family (no history of bleeding and thrombosis) were compatible with molecular results since Tokyo II fibrinogen does not have thrombotic and hemorrhagic nature and lack of clinical signs in
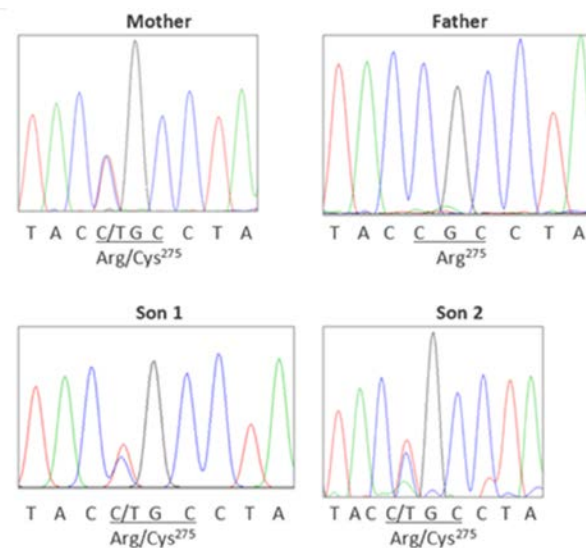
Fig. 1. Detection of Arg275Cys mutation of γ-fibrinogen in three affected members. Heterozygous mutation was shown in codon 901(CGC to TGC) in mother and her affected sons.

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Acknowledgment

Conflicts of Interest

The authors declare that they have no conflict

of interest.

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