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Factor XIII Deficiency in Three Iraqi patients with a Variations in Gene F13A1 Detected by Next Generation Sequencing

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Abstract

Factor XIII (FXIII) plays a crucial function in the coagulation process by stabilizing the hemostatic clot. FXIII deficiency is linked to a higher susceptibility to bleeding, with dominance of approximately one case per two million individuals in the general population.The most common causes of FXIII deficiency result from a genetic variation in FXIII- A1 gene. The aim of this study is Identifying the variations of FXIII gene among Iraqi patients through whole Exome Sequencing using Next Generation Sequencing Technology of the Proband is the major objective of this work. Three variants were detected among three samples, involved: c.1814 T>C (p. Leu605pro; NM_00129.4),c.1478T>A (p. Leu493Ter; NM_00129.4), and c.1113-1G>A, NM_000129.4), located in the F13A1 gene. Also, in one of the samples A variation was observed in two other genes called CD36 (c.1079T>G (p. Leu360Ter NM_001001548.2), and DDX58 gene c.1527 T>G (p. Tyr509Ter; NM_ 014314.4). The all detected variant in F13 A1gene been in homozygosis and parents in heterozygosis.

Keywords: *Inherited Rare Bleeding Disorder, FXIII deficiency, Coagulation Disorders, Whole Exome Sequencing.*

Introduction

Fibrin stabilizing contributor which is the name of the coagulation factor-XIII(FXIII) it is a vital role in the coagulation process (1). FXIII is a part of the transglutaminases family and circulating as a heterotetrametric form, made up of a couple of catalytic subunits referred to as factor XIII subunit A (factor XIIIA) and two carriers subunit known as factor XIII subunit-B (factor XIIIB) (3).

During fibrinolysis, factor XIII-A triggers the activation of anti-plasmin that is resulting in the cross linking of fibrin molecule to enhance the clotting steadiness [4]. Also, it inhibits the degradation of the cross linked fibrin structures by plasmin (5). Consequently, factor XIII-A assumes a significant role in diverse processes, such as extracellular remodeling, atherosclerosis, angiogenesis, wounds recovery, cell adhesion, migration, and tissues repairs besides role in coagulation and fibrinolysis (6).

On the other hand, FXIII-B primarily shields FXIII-A2 from degradation andinactivation, there by prolonging its circulation time. Additionally, FXIII-B aids in FXIII localizing in order to polymerize fibrins series facilitating the crosslinking process. FXIII-B is primarily produced in the liver (7) and, once in the plasma, it selectively attaches to

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the γ' chains of fibrinogen type 2 acting as a transporter of FXIII and regulating its ability to crosslink. In contrast, FXIII-A is synthesized by bone marrow cells (8).

The gene responsible for the encoding of the FXIII-A submits is consisting of fifteen exon spanning a genomic area of 160 kb, located on the 6p24-25 chromosome area. The FXIII-A subunit involves 731 amino acid besides it is known by 5 regions: β-sandwich (residues 38-183), β-barrel 2 (residues 628-731) the central domain of the catalytic core region (residues 184-515), the activation peptide (residues 1-37) β-barrel 1 (residues 516-627) (9).

Hereditary Bleeding Disorder (HBDs) encompass a number of inherited abnormality issues affecting the first and the second types of homeostasis. These disorders arise from deficiencies or functional abnormalities of plasma protein incorporated in normal coagulations (10). Inherited bleeding disorder might be leading to bleeding for lifelong and are primarily attributed to the qualitative/quantitative defects in coagulations contributors (11). One such condition is Congenital FXIII deficiency,a significant and scarce bleeding disorder with an approximated prevalence of approximately 1 in 2 millions in the whole population (10) and 111 reported cases in Iraq (8). This disorder is inherited as autosomal recessive based recession diseases, being higher in countries where consanguineous marriages are diffused (16). The Consanguineous marriages are marriages contracted between blood relatives or union between two people who share an ancestor (20).

Factor XIIIA deficiencies is predominantly associated with mutation of FXIIIA genes, which accounts for approximately 95% of instances. Homozygous mutation typically results in reactions associated to the mal functioned blood coagulations, while sick people having heterozygous mutation usually do not exhibit any reactions (11). Clinically based manifestation of FXIII deficiency consists of bleeding in subcutaneous, delayed wound healing, frequent abortion and miscarriages Umbilical Cord Bleeding (UCB), and soft tissue. Intracranial bleeding is the primary cause of death, and the most severe hemorrhage cases are observed in people with a significant deficiency (12). Once of molecular methods uses for detect genetic variation is next generation sequencing, Next-Generation Sequencing (NGS), also known as high-throughput sequencing, one of this technologies is Illumina (Solexa) sequencing (18).

The major objective of this study is to identify genetic variations in three Iraqi patients with factor XIII deficiency through whole genome exome analysis of the F13A1 gene using NGS technology, contributing to a deeper understanding of the defects in this coagulation factor.

Materials and Methods

The research included three patients from distinct families who had FXIII deficiency. These individuals exhibited bleeding tendencies, even though their standard coagulation tests were normal. The diagnoses were affirmed by conducting the clotting solubility test in 5M urea at the Hemophilia Ward, Children Welfare Teaching Hospital, Medical City, Baghdad.

The first sample was a 22-year-old female, while the other two samples were males, 26 and 11 years old. The three patients families were distinguished by the relatives between their parents and affected the offspring. Each family had two affected children.

The genomic DNA of the three samples was separated out of the entire blood specimen on the basis of theReliaPrep™ Blood gDNA Miniprep System, Promega protocol. Quantus Fluorometers were utilized for detecting the concentrations of extracted DNA fordetecting the samples quality.

Extracted DNA was successfully amplified in a multiplex PCR, followed by tagging with sample-specific indexes and sequencing adapters. The mean read length was 240 base pairs and average target coverage of >600x was achieved, with 95.5% of target bases covered more than 100x. In an effort to find variants associated with factor 13 deficiency,Whole Exome Sequencing using Next Generation Sequencing Technology of the proband. Checked F13-A1 gene associated with reduced Factor XIII activity. Also, was further investigated 78 genes associated with bleeding disorders. As well as possible autosomal recessive causes and regions of homozygosity.

The variants were evaluated based on their pathogenicity and causality, following the guidelines recommended by American College of Medical Genetics (ACMG). All variants related to the patients' phenotype were reported, excluding benign or likely benign variants. The effects of the variants were assessed using mutation taster in silico database predictions.

The sequences obtained from NGS sequencing machines are aligned to a human reference genome (GRCh37/hg19) and variants were called using Illumina Dragen germline pipeline (ver. 01.011.332.3.4.5). The variants detection was restricted to the targeted region covered by the library preparation kit. Recommended filters were applied to ensure the quality of the results was on par with expected QC metrics.

Every identified variation is thoroughly assessed for its potential to cause disease and its causal relationship. Following the ACMG guidelines, these variants are classified into classes 1 – 5. All variants that have a connection to the patient's characteristics are reported, except for those considered benign or likely benignvariants, are reported. The effected of variants was diagnostic by mutation taster in silico database predictions.

Results

The results of genetic disorder were detected by Whole Exome Sequencing using Next Generation Sequencing Technology of the proband and patients' characteristics for the three patients' samples are resumed in the following table-1. The FXIII deficiency was diagnosed in the one index patient, male 11 years old. From family have three children two males and one female, one of the boys and sister were affected with F13 deficiency. The other patient's sample is 22 years old female from a family having five children threemales and two females, one of the two girls and one of the boys are affected with factor XIII deficiency. The third patient's sample is 26 years old male, from a family having three children, two males and one female. The two sons are affected with factor XIII deficiency.

Table-1:Spectrum of Factor XIII-A Subunit Gene Mutations in three Iraqi Patients with Factor XIII Deficiency.

Patients no.	**Gene ID**	**Location**	**Variant**	**Zygosity**	**Disorder (Omim Ref#)**	**Inheritance**	**Variant classification**
P1	**F13A1 NM_000129.4**	Exon 12	c.1478 T >A p. Leu493Ter	Homozygous	- Factor XIII, A subunit, Deficiency of -Thrombophilia due to Thrombin Defect;THPH1	- Autosomal Recessive - Autosomal dominant	uncertain significance (Coverage 0:58)

	CD36 NM_001001548.2	Exon 11	c.1079T>G p. Leu360Ter	Homozygous	- PlateletGlycoprotein IVDeficiency	- Autosomal recessive	uncertain significance (Coverage 0:50)
	DDX58 NM_014314.4	Exon 11	c.1527T>G p. Tyr509Ter	Heterozygous	- Singleton marten Syndrome2;SGMRT2	- Autosomal dominant	uncertain significance (Coverage 16:19)
P2	**F13A1 NM_000129.4**	Exon 13	c.1814T>C p. Leu605Pro	Homozygous	- Factor XIII, A Subunit, Deficiencyof -Thrombophilia due to Thrombin Defect; THPH1	- Autosomal Recessive - Autosomal dominant	uncertain significance (Coverage 0:157)
P3	**F13A1 NM_000129.4**	Intron 8	c.1113 1G>A	Homozygous	- Factor XIII, ASubunit, Deficiencyof -Thrombophilia due to Thrombin Defect; THPH1	- Autosomal Recessive - Autosomal dominant	uncertain significance (Coverage0:81)

Discussion

The results of the ExoSeq technique in the first family showed the present of genetic variation in some genes causes bleeding tendency. The first variation in coagulation F13 A1 gene c.1478T>A (p. Leu493Ter; NM_00129.4) within exon 12, this variant was classified as uncertain significance in a homozygous state and exhibits an autosomal dominant and autosomal recessive inheritance pattern. However, Mutation Taster in silico database predicts it to be disease causing. Furthermore, no publications or gnomAD population data exists for this variant. Therefore, it is unknown how this novel mutation exists in certain population groups and which effect it might causes.However, this variant is a stop gain variant which results in a premature termination codon which mightinterrupts the functionality of the protein. It shows good coverage and quality and appearsin various gene and phenotype filters that were applied.

Other variations were detected for the same patient in c.1079T>G (p. Leu360Ter; NM_001001548.2) was homozygous autosomal recessive and causes a deficiency in platelet glycoprotein that plays a key role in hemostasis by interacting with extracellular matrix and c.1527T>G (p. Tyr509Ter NM_014314.4) that cause Singleton-Merten syndrome-2.

Second patient was homozygous for variant c.1814T>C p. Leu605Pro (p. Leu 605 Pro NM_000129.4) in exon 13 of F13A1 gene (table-2).This variant (c.1814T>C) is classified as a variant of uncertain significance by ClinVar. However in silico databases SIFT, PolyPhen2 and Mutation Taster predicts it to be deleterious, probably damaging and disease causing. No publications or gnomAD population frequency exists for this variant. Hence, the presence and implications of this novel mutation in specific population groups remain uncertain. Nonetheless, it is worth noting that another disease-causing variant (HGMDCM078784) exists at the same position, and the protein seems to be conserved among various species. It shows good coverage and quality and appears in various gene and phenotype filters that were applied. Furthermore, it explains the clinical features described in this patient and may confirm the clinician's diagnosis.

The index patient of the third family was found to have a genetic variation in intron 8 (c.1113 1G>A; NM_000129.4) that causes deficiency in coagulation factor XIII. A homozygous variant (c.11131G>A) of uncertain significance was observed in the F13A1 gene in one sample from the three patients. This variant is classified as a VUS (Variant of Uncertain Significance); however, it has been suggested to be a deleterious variant by in silico analysis (Mutation Taster). The variant is located at the first intronic nucleotide, right at the exon-intron boundary, and may adversely affect mRNA splicing, leading to a defective mRNA and potentially abnormal protein production. All three samples were found to have the homozygous mutant, and the symptoms of the disorder were diagnosed in the first months after birth.

Factor 13 A1 deficiency is a single-gene autosomal recessive inheritance pattern disease, that differ from other bleeding disorder been X-linked inheritance such as Haemophilia B (19) and it was detected in a consanguineous family due to mandatory migration that obtained a 25% occurrence when both parents are carriers (heterozygotes). The parents (heterozygotes) tend to be asymptomatic (12). Consanguineous marriage is a potential risk factor for an increase in autosomal recessive traits with a strong effect, leading to the appearance of recessive disorders (16). Molecular analysis explains that about 95% of those affected have Factor XIII-A deficiency, mainly caused by mutations in the FXIII-A gene (13). This is similar to the obtained result which links the disease with variation in factor XIII-A. In other cases, the deficiency may be caused by variations in factor XIII-B subunit or may be related to autoimmune disease (13). More studies have identified wide genetic variations in factor XIII-A related to factor 13 deficiency in different areas, in Iran diagnostic molecular analysis found five single-nucleotide polymorphisms (SNPs) in different exons 4, 6, 9, 10, and 15 (12). The variant Val34Leu is one of the most common polymorphisms in factor XIII deficiency (17); however, this variant wasn't detected in the three Iraqi patient samples.

Conclusion

Three patients from three different families with FXIII deficiency were diagnosed based on bleeding tendencies and normal standard coagulation tests. Whole exome NGS technique was used to screen all the coding boundary regions of the F13A1 gene in these three affected patients. Three variants were identified: exon 13 c.1814T>C (p.Leu605Pro; NM_00129.4), exon 12 c.1478T>A (p.Leu493Ter; NM_00129.4), and intron 8 c.1113-1G>A (NM_000129.4), all located in the F13A1 gene. Additionally, one patients sample showed detected variants in the CD36 gene c.1079T>G (p.Leu360Ter NM_001001548.2) and DDX58 gene c.1527T>G (p.Tyr509Ter; NM_014314.4).

The genetic and molecular investigations aligned with the functional investigations and clinical observations, supporting the findings. Given the elevated risk of severe bleeding and early symptoms in individuals with significant FXIII deficiency, it is crucial to promptly confirm the genetic basis. Next-Generation Sequencing (NGS) technology enables swift and simultaneous examination of all relevant gene regions associated with the condition.

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Ethical Clearance

This research was ethically approved by the Research Ethical Committees of the Ministry of Environmental and Health and the Ministry of Higher Education and Scientific Research, Iraq.

Abbreviations

FXIII	Factor XIII
HBDs	Hereditary Bleeding Disorder
Factor XIIIA	Factor XIII subunit A
Factor XIIIB	Factor XIII subunit B
UCB	Umbilical Cord Bleeding
NGS	Next Generation Sequencing
PCR	Polymerase Chain Reaction
ACMG	American College of Medical Genetics

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