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Research Article

Factor XIII Gene Polymorphisms among Sudanese Patients with Intracerebral Hemorrhage, Khartoum State 2022

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Abstract

Background: Intracerebral hemorrhage (ICH) is a sudden bleeding into the tissues of the brain, into its ventricles, or into both. It is the second most common subtype of stroke and is a critical disease usually leading to severe disability or death.

Material and method: This study was a cross sectional hospital-based study, conducted at the research laboratory of the national center of neurological sciences (NCNS), Khartoum, Sudan during the period June 2022 to August 2022.It included all patients attended with intracerebral hemorrhage. DNA extraction was done from blood of all patients and control. PCR for *factor XIII gene* was carried out and thus Sanger sequencing to both cases and controls.

Results: The PCR results showed; 100% samples were positive for *factor XIII gene*.Sequencing result showed the detection of threepolymorphisms in factor *XIII gene* (G>T, A>G and C>T) .

Conclusion: The detected factor XIII gene polymorphisms (G>T, A>G and C>T) might be associated with intercereberal hemorrhage among Sudanese patients.

Keywords: Factor XIII, gene, polymorphism, hemorrhage, stroke, cerebrovascular

INTRODUCTION

Intracerebral hemorrhageICH, the second most frequent type of stroke after ischemic stroke, is responsible for a disproportionately high rate of cerebrovascular death and morbidity. It is a severe disorder when a hematoma forms in the brain parenchyma with or without blood extension into the ventricles. It is one of the subtypes of stroke. [1]

Chronic hypertension, amyloid angiopathy, anticoagulation (medication), and vascular abnormalities are risk factors for ICH. The resulting brain damage is frequently divided into two categories: primary, or the damage brought on by the blood clot's initial damage to the parenchyma, and secondary, or the damage brought on by its consequences. [2]

The most significant risk factor for spontaneous ICH is hypertension, which has a bigger impact on deep ICH than on lobar ICH. Smoking is the most prevalent modified risk factor, and there are other more risk factors, including coagulapathy. [3,4,5]

Blood from humans and some other animals contains a zymogen known as factor XIII, also known as fibrin stabilizing factor. Factor XIIIa is produced when thrombin activates it. Fibrin in coagulation system is crosslinked by the active enzyme factor XIIIa. Clot stability is worsened and bleeding tendency is increased by XIII deficiency. In the human blood, factor XIII is found as a heterotetramer composed of two A and two B subunits. The B units on the clot are where factor XIII attaches. Thrombin effectively breaks the R37-G38 peptide

link of each A unit in an XIII tetramer when fibrins are present. The N-terminal activation peptide of A units is released.[6]

In humans, the most important proteolytic inhibitors of the active factor XIIIa are plasmin, antithrombin, and tissue factor pathway inhibitor TFPI. A prominent non-proteolytic inhibitor is 2-macroglobulin. On chromosomes 6 and 1, two distinct genes, one with a length of more than 200 kb and 15 exons, and the other with a length of 28 kb and 12 exons, respectively, encode the XIII A and B subunits.[6]

Our review of the literature indicates that there are 51 sequence alterations in the *FXIII-A gene* and three in the *FXIII-B gene*, which together form the genetic basis for factor XIII deficiency. Plasma of FXIII G103T variant carriers was reported to activate FXIIIA by thrombin two to three folds more quickly. Because the catalytic effectiveness of thrombin-induced cleavage of FXIIIA changes the structure of the cross-linked fibrin, the product of fibrin fully cross-linked by FXIII G103T has a finer structure with thinner fibers and smaller pores, which has an impact on clot stability. As a result, fibrin fiber lateral aggregation is compromised. [7]

Genetic variations and their connection to ICH are the subject of debates among studies from all over the world. The proposed panel of genetic variations has not been the topic of any published studies in Sudan. In this study, we will look for potential polymorphisms in the factor XIII gene among Sudanese patients with ICH.

MATERIAL AND METHODS

A cross sectional hospital-based study was conducted at the research laboratory of the national center of neurological sciences (NCNS), Khartoum, Sudan during the period June 2022 to August 2022.

All patients attended NCNS and diagnosed with ICH during the aforementioned period were included. In addition to that, apparently healthy individuals with no history of ICH were selected as control group.

From each participant 3 ml of venous blood was collected from the antecubital vein using a dry sterile disposable syringe and needle. Blood samples were dispensed into sterile containers with Ethylene Diamine Tetra-acetic Acid (EDTA), label with subject's age, sex and identification number and were stored at -20°C for molecular analysis.

The Genomic DNA isolated by G-DEX IIB Genomic DNA extraction Kit.Primers were designed by using Prime3 software. The forward primer for FXIII Val34Leu(G-T) was designed as "5-CATGCCTTTTCTGTTGTCTT-3"and reverse as "5-GTTGACGCCCCGGGGCACTA-3"with product size of 192bp fragment.

For polymerase chain reaction PCR:Double distilled water (14 ul) wasplaced in PCR tube, then 4 ul of master mix, 1 ul of forward primer, 1 ul of reverse primer and 2 ul of DNA sample was added then vortexed. The PCR tube containing this mixture wasplaced in commercial thermal cycler (Swift™MaxPro SWT-MXP-BLC-4)atfollowing condition: Denaturation temperature 94°C for 30 secs, annealing temperature at 61°C for 30 sec and extension temperature at 72°C for 30 secs, the final elongation was adjusted for 5 minutes at 72 °C.PCR reaction was set at 35 cycles.The PCR amplification product was separated on agarose gel products were sent for sequencing to Macrogene Europe Laboratory.

Data was entered and organized into Microsoft Office Excel 2010 data sheet, then for the analysis, SPSS version 23 statistical software (SPSS Inc., USA) was used for statistical analysis. Data were expressed as means with standard deviations (SD). The statistical analysis was performed by the analysis of variance. A value of P <0.05 was considered statistically significant.

The study was approved by the ethical committee of the National Center for Neurological Sciences and ethical review committee of the National University, faculty of medical laboratory, and the participants was fully informed about the advantages and disadvantages before participation in the research (verbal informed consent).

RESULTS

A total of 100 participants were enrolled in this study, 50 were selected as cases and 50 were selected as control group.

In the case group; 64% were male and 36% were female, the most affected age group more than 70 years (38%), followed by less than 50 years and 50-70 years (34%, 28%) respectively. Most of them were from Khartoum state (66%). About 42% of the cases had no history of chronic disease, 26% had a hypertension and 24% were diabetic. Regarding the types of cerebral Hemorrhage; 64% had a subdural hemorrhage and 30 % had intercereberal hemorrhage. (Table 1,2,3)

Table 1: Socio-demographic data of the cases

Sociodemographic		Frequency	Percent
Gender	Male	32	64.0
	Female	18	36.0
	Total	50	100.0
Age	< 50 years	17	34.0
	50 - 70 years	14	28.0
	> 70 years	19	38.0
	Total	50	100.0
Place of origin	Khartoum	33	66.0
	Aljazirah	10	20.0
	White Nile	1	2.0
	River Nile	2	4.0
	Kassala	1	2.0
	Korodofan	3	6.0
	Total	50	100.0

Table 2: Distribution of the Chronic Disease in the Case Group

Chronic Disease	Frequency	Percent
Hypertension	13	26.0
Diabetic	12	24.0
Diabetic & hypertension	4	8.0
Absent	21	42.0
Total	50	100.0

Table 3: distribution of cerebral hemorrhage in the case group

Hemorrhage	Frequency	Percent
ICH	15	30.0
SDH	32	64.0
EDH	2	4.0
IVH,ICH,EVD	1	2.0
Total	50	100.0

Molecular outcomes revealed the detection of 192 bp of *factor XIII gene* in gel electrophoresis for both cases and controls (Figure 1).The sequencing results were analyzed using different bioinformatics soft-wares and tools. For the examination of thepresence of polymorphisms theobtained sequences, were aligned using BioEdit-ClustalW software with a normal sequence from GenBank (National Center of Biotechnology Information).When the cases were compared with the normal reference the following single base exchange were found G>T, C>T, and A>G. While when the controls were examined, no difference was among controls group. (Figure 2,3)

Mutation taster was used to confirm the mutations. It revealed; G>T base exchange polymorphism prediction, amino acid sequence was change, protein features might be affected and splicing site changes were shown (Figure 4).

Regarding the C>T base exchange, polymorphism was predicted using the mutation taster, amino acid sequence was changed, protein features might be affected, splice sits changes alteration location was expected at chromosome 6, alteration type was single base exchange, cDNA changes position was 277. (Figure 5)

Moreover, A>G base exchange was checked by mutation taster. Polymorphism changeprediction revealed, amino acid sequence change, protein features might be affected and splicing site also was changed, alteration location was at chromosome 6, cDNA changes position was 273, alteration type was single base exchange.(Figure 6)

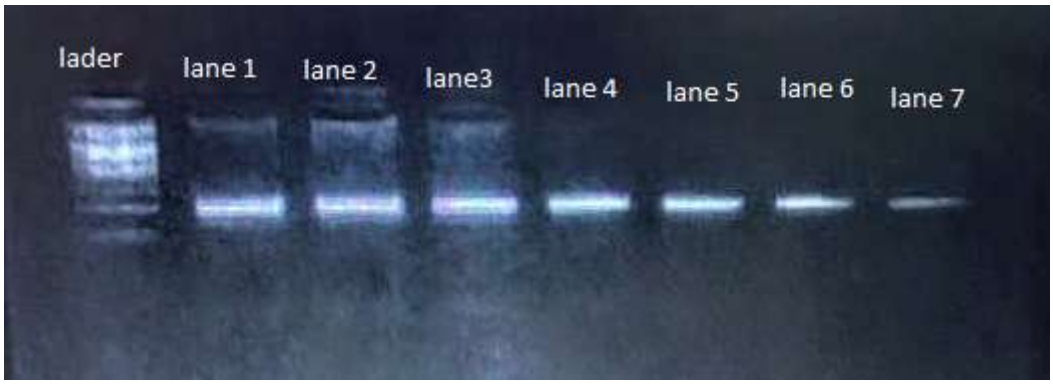

Figure 1: *Factor XIII gene*(192 bp) detectionin gel electrophoresis in cases and controls

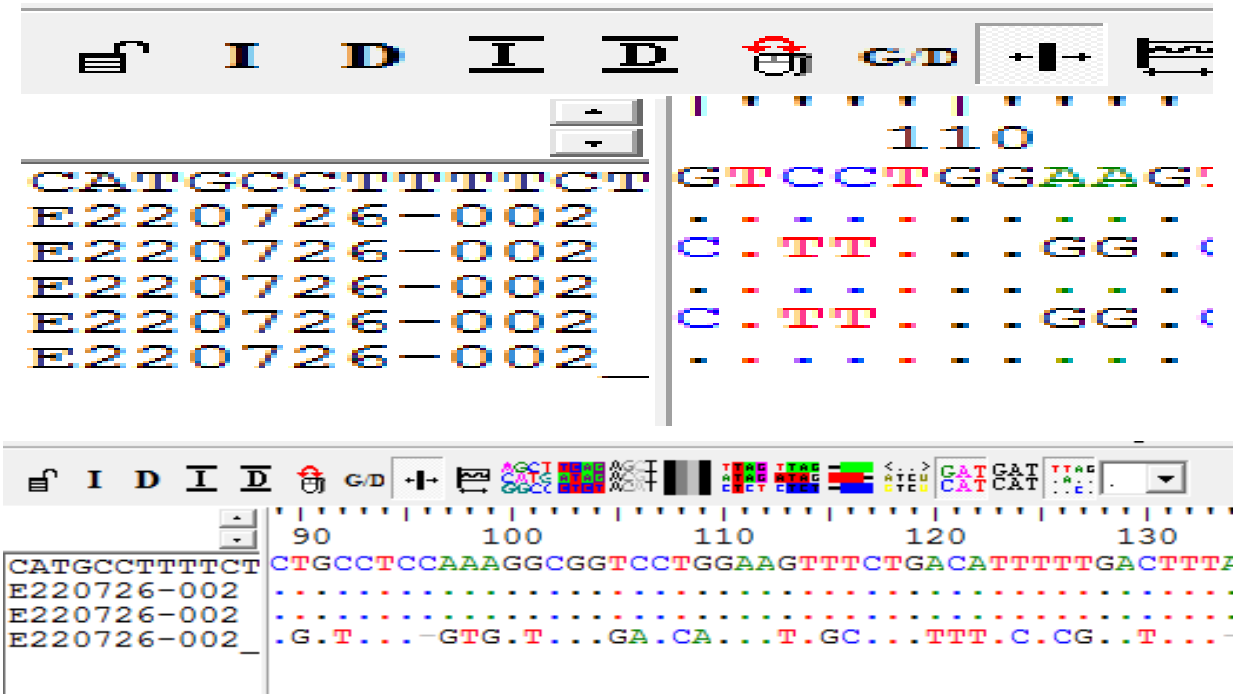

Figure 2: Multiple sequence alignment using Bio-Edit clustal W for cases group with reference gene sequence of *F XIII gene.*

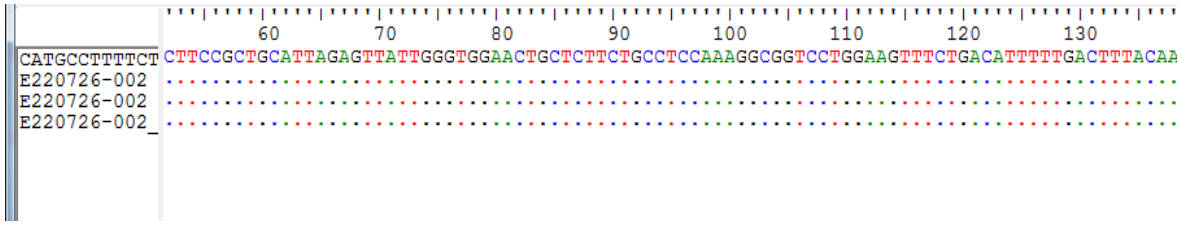

Figure 3: Multiple sequence alignment using Bio-Edit clustalW for control group with reference gene sequence of *F XIII gene*

Prediction **polymorphism** Model: *without_aae*, prob: 0.999999726302253 (explain)

snippet not found in sequence; wild-type and mutated allele were switched

Summary hyperlink

- **protein features (might be) affected**
- **splice site changes**

analysed issue	analysis result
name of alteration	no title
alteration (phys. location)	chr6:6248388A>C/A show variant in all transcripts IGV
HGNC symbol	F13A1
Ensembl transcript ID	ENST00000264870
Genbank transcript ID	NM_000129
UniProt peptide	P00488
alteration type	single base exchange
alteration region	intron
DNA changes	g.72859T>G
AA changes	N/A
[illegible]	N/A
frameshift	N/A
known variant	Variant was neither found in ExAC nor 1000G. Search ExAC
regulatory features	N/A
phyloP / phastCons	PhyloP PhastCons (flanking) 1.37 0.006 0.317 0.003

Figure 4: Predictions of G>T singles Base Exchange tested in mutation taster application

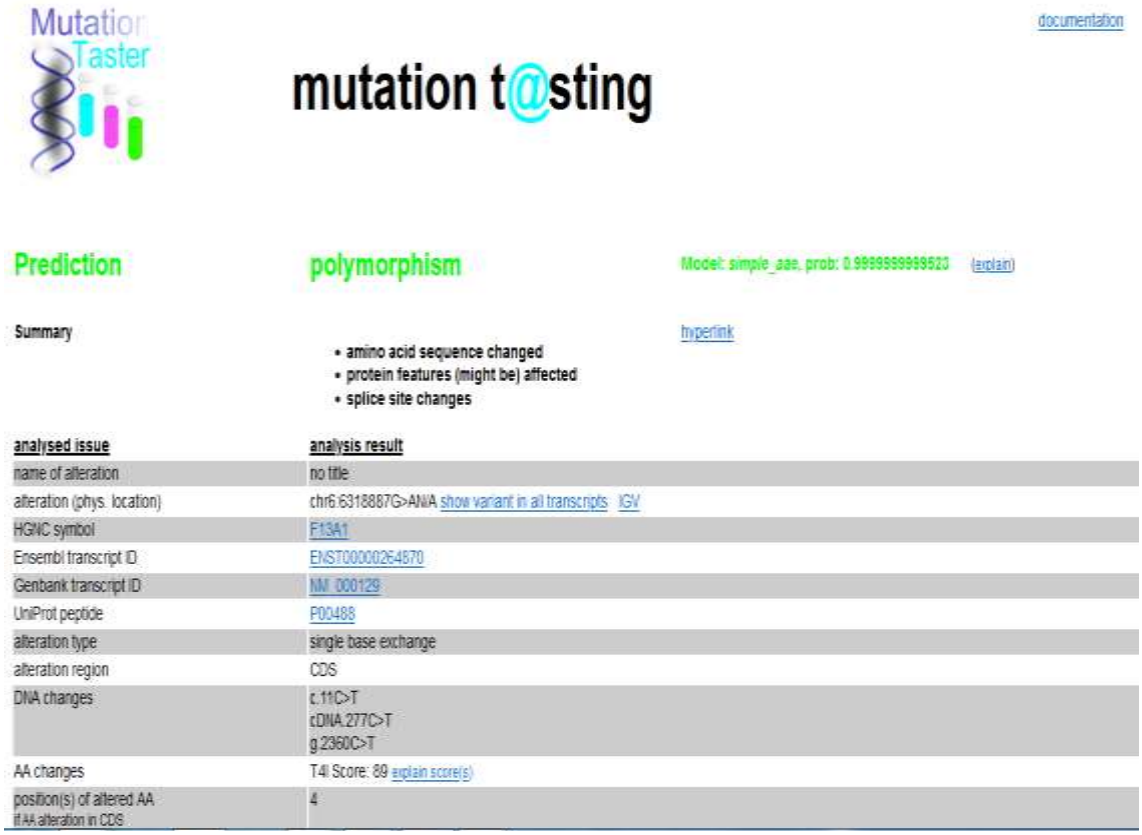

Figure 5: Prediction of C>T singles base exchange tested in mutation taster application

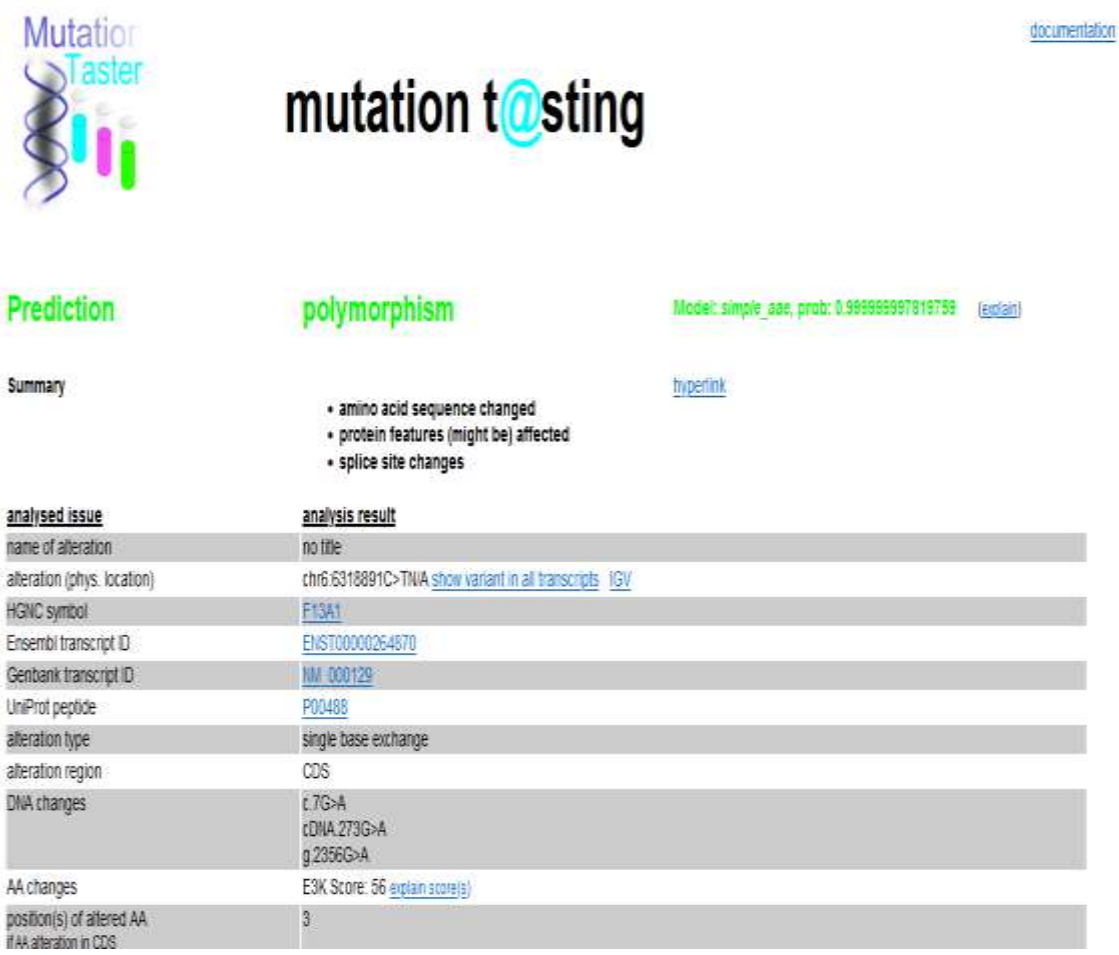

Figure 6: Prediction of A>G singles Base Exchange tested in mutation taster application

DISCUSSION

In the last few decades, there has been a fast advancement in the epidemiology of spontaneous intracerebral hemorrhage (ICH), thanks to significant genetic and nongenetic findings. [8]

An investigation of the age of ICH conducted in the UK revealed that since the early 1980s, effective hypertension management has resulted in a decrease in the incidence of ICH linked with hypertension in individuals under the age of 75. However, a proportion of ICH cases continued to be stationary, which was most likely caused by an increase in non-hypertensive lobar ICH in the current research; ICH does not emerge at a certain age. Numerous research have examined the epidemiology of this terrible illness, but it is difficult to quantify how gender affects incidence and fatality. [9]

Males were found to be two times more numerous than females, according to our results on gender disparity, but there is still no information on the biological differences in this situation.

ICH and hypertension have long been linked, and current research shows that untreated hypertension significantly raises the chance of developing ICH[10,11]

Anticoagulant therapy, which is frequently administered to patients with hypertension and type II diabetes, has also been consistently linked to an increased risk of intracranial hemorrhage (ICH), particularly cerebellar ICH. [12]

Nearly half of the patients in the current study had chronic hypertension, although the majority of our patients did not have either diabetes or hypertension. In addition, the Val34Leu polymorphism of the *Factor XIII gene* has been linked to increased Factor XIII activity. Inherited deficiency of the proenzyme Factor XIII is a very rare autosomal-recessive bleeding condition with a notably high incidence of cerebral hemorrhage. Additionally, researches linking this polymorphism to ICH have produced contradictory results. [13]

In addition, the most frequent polymorphism affecting the *FXIII gene* results in a valine (V)-to-leucine (L) substitution at amino acid 34 (FXIII G103T) in exon 2 of the gene encoding for FXIIIA. FXIII plasma concentration is unaffected by the G103T variation, although the amino acid shift may alter FXIII activity.

Results from the current investigation were examined utilizing a variety of bioinformatics software and techniques. When the examples were compared to the standard reference, the single base exchanges G>T, C>T, and A>G were discovered. When the controls were compared to the typical reference, no base exchange at all was discovered in any of the control groups. The three mutations were confirmed using a mutation taster, which showed that they were anticipated to be Base Exchange Polymorphism, amino acid sequence alterations, potential effects on protein characteristics, and changes to splicing locations.

A common point mutation (G=T) in exon 2 of the factor XIII a-subunit gene is protective against myocardial infarction2 and predisposes to intracerebral hemorrhage, according to Catto et alstudy's which also revealed that patients with intracerebral hemorrhage had an excess of the factor XIII Leu allele. [14] These results provide credence to the idea that factor XIII 34Leu contributes to the creation of less robust fibrin structures, which may protect against blood clot formation while increasing the risk of bleeding. [15]

Additionally, Tu CQ et colleagues reported that the risk of ischemic cardiovascular and cerebrovascular disorders was related with the G > T (p.Val35Leu) polymorphism. The G > T (p.Val35Leu) polymorphism has been linked to an increased risk of intracranial hemorrhage and brain infarction in Caucasian populations. [16,17]

Exon 12's C > T polymorphism influences the enzyme's particular activity. Additionally, the C > T mutation lowers plasma F13A levels while raising F13A activity. [18] The polymorphism was predicted in the current investigation, and this revealed a clear connection between the pathogenicity of the disease and the presence of polymorphism. This is consistent with the findings of Arati et al., who found that the polymorphism 1694C > T (p.Pro564Leu) exhibited a substantial connection with risk of ICH.

In addition, they noted that the 564Leu allele was linked to a higher risk of SAH in the South Indian population, whereas the 34Leu allele was protective for a lower risk of the condition. [19]

Additionally, no research that looked into the relationship between the FXIII A>G polymorphism and ICH could be discovered in the literature. Our research showed that these polymorphisms altered the amino acid sequence, which may have an impact on the characteristics of proteins. Additional research is required on these polymorphisms.

CONCLUSION:

The detected factor XIII gene polymorphisms (G>T, A>G and C>T) might be associated with intracerebral hemorrhage among Sudanese patients, because it may lead to production of weaker fibrin structures, and might thereby protect against clot formation and predispose intracerebral hemorrhage.

Large samples would be enrolled in mega analysis study, and should include other coagulation factors so as to predict the recovery period among patients with this hemorrhage, and eventually the improvement of neurological deficit.

Competing Interests

The authors declare that they have no competing interests.

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