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Research Article,

Prothrombin Gene Polymorphism (G20210A) Among Sudanese Patients with Intracerebral Hemorrhage, Khartoum State, 2022

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Abstract:

Background

Intracranial hemorrhage is the third most frequent cause of cerebrovascular disease, also known as cerebral bleed, intraparenchymal bleed and hemorrhagic stroke. This study was designed to detect the possible present of factor II polymorphism (G20210A) among Sudanese patients with Intercereberal hemorrhage.

Material and method

This study was 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, demographic data (age , gender, associated disease). DNA extraction was done from blood of all patients and control.PCR for factor *prothrombin gene* was done and thus Sanger sequencing.

Results:

The PCR results showed; 100% positive for *factor II gene*. And sequencing result showed base bare exchange in factor II gene G to A (G20210A) polymorphism.

Conclusion

Factor II gene polymorphism (G20210A) was detected and might be association with Intercereberal hemorrhage among Sudanese patients

Keywords: Monkeypox, Outbreaks, WHO, Zoonotic disease, United States

Introduction:

Intracranial bleeding is the third most common cause of cerebrovascular disease after cerebral bleed, intraparenchymal bleed, and hemorrhagic stroke.It is also the second most prevalent subtype of stroke and a serious condition that frequently results in severe impairment or death. Hypertensive vascular disease is one of the most frequent causes of intracerebral hemorrhage, with basal ganglia hemorrhage accounting for 80% of cases in hypertensive people over the age of 50. [1]

Approximately 10–20% of all strokes are caused by the incidence of ICH, which is 8–15% in western nations like the USA, UK, and Australia and 18–24% in Japan and Korea. The incidence rate of ICH per 100,000 person-years was 51.8 in Asians. With advancing age, ICH incidence rises. Importantly, hemorrhagic stroke was discovered to be the cause of 41.6% of stroke cases in Sudan. Stroke is a condition that carries a high risk of morbidity and fatality. [2,3]

While these risk factors account for a sizable amount of the diversity in ICH risk, a sizable portion of this variation is still unaccounted for. Furthermore, heritability estimates based on genome-wide data from unrelated individuals suggest that up to 30% of ICH risk can be explained by common and rare genetic variation. Population genetics can help to resolve these two outstanding concerns. [4]

The epsilon variants of *apolipoprotein E (APOE) gene* have been noted as a significant genetic risk factor for ICH in one of the Candidate Gene investigations. Along with *ACE, COL4A2, 1q22, TIMP1, TIMP2, MMP2, MMP9, and TNF genes*, this is related with lobar ICH (LICH), while *GPX1, CR1, ITGAV, PRKCH, and 12q21.1 genes* are connected with deep ICH (DICH). [5]

Coagulation Factor II

Glycoprotein, a carbohydrate-protein complex with a molecular weight of roughly 68,000, is a protein produced by the liver (vitamin K dependent). A vital element of the blood clotting process that is present in blood plasma. It is a -globulin and has a concentration of 300 lowa units/ml, or 15 g/ml, in plasma. [6]

A crucial element of the blood coagulation pathway is thrombin. It is the starting point for the serine protease thrombin, which has fibrinolytic, anti-fibrinolytic, and pro-coagulant properties. The *prothrombin gene* is found at 11p11-q12 on chromosome 11. It is 21 kb in length and has 14 exons as well as regulatory non-coding sequence. [7]

The enzymatic cleavage of two prothrombin sites by activated Factor X (Xa) results in the production of thrombin. Binding to activated Factor V (Va), also known as the prothrombinase complex, significantly increases the activity of factor Xa. [8]

Thrombin converts factor XI to XIa, factor VIII to VIIIa, factor V to Va, fibrinogen to fibrin, and factor XIII to XIIIa in the blood coagulation pathway. The cleavage of fibrinopeptides A and B from the corresponding A and B chains of fibrinogen to create fibrin monomers is catalyzed by thrombin during the conversion of fibrinogen into fibrin. [9]

Including genetic variations and their association with ICH, there is a substantial disparity between studies conducted around the world. Some polymorphisms that alter clotting factors have recently been linked to increased risk of thrombosis and bleeding, according to a few studies. Reports that have examined how polymorphisms affect the hemostatic state in bleeding diseases, however, have not done so sufficiently.

The list of hypothesized genetic variants was not included in any studies that were published on this subject in Sudan. Therefore, the goal of this investigation was to identify Sudanese individuals with ICH who had the *factor II gene* polymorphism (G20210A).

Materials and methods:

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

The data was collected using pre-designed structural questionnaire; the demographic and clinical data concerning each participant was obtained from the registry data base office, which included the following information :(Gender, age and medical history). The laboratory data included hematological results, PCR findings and sequencing results.

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

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

Genomic DNA was isolated using G-DEX IIB Genomic DNA extraction Kit. Primers were designed by using Prime3 software. The forward primer for FII (G20210A) was designed as "5-TCTAGA AAC AGT TGC CCT GGC -3"and

reverse as "5- ATA GCA CTG GGA GCA TTG AAGC -3" with product size of 192bp fragment.

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 vortex. The PCR tube containing this mixture wasplaced in commercial thermal cycler (Swift^{TM}MaxPro SWT-MXP-BLC-4) at following condition: Denaturation temperature 94^{o}C for 30 secs, annealing temperature at 61^{o}C for 30 sec and extension temperature at 72^{o}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 Macro gene 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 was 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.

Sequencing analysis:

The sequencing results were analyzed using different bioinformatics soft-wares and tools.The obtained sequences, aligned using BioEdit-ClustalW software with a normal sequence from GenBank (National Center of Biotechnology Information), was examined for the presence of polymorphisms.

Results:

The epidemiological study: 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 was 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%). In addition to that about 42% hadn't history of chronic disease, 26% had a hypertension and 24% were diabetic. For the types of cerebral Hemorrhage; 64% had a subdural hemorrhage SDH 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 living	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 Associated Diseases 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

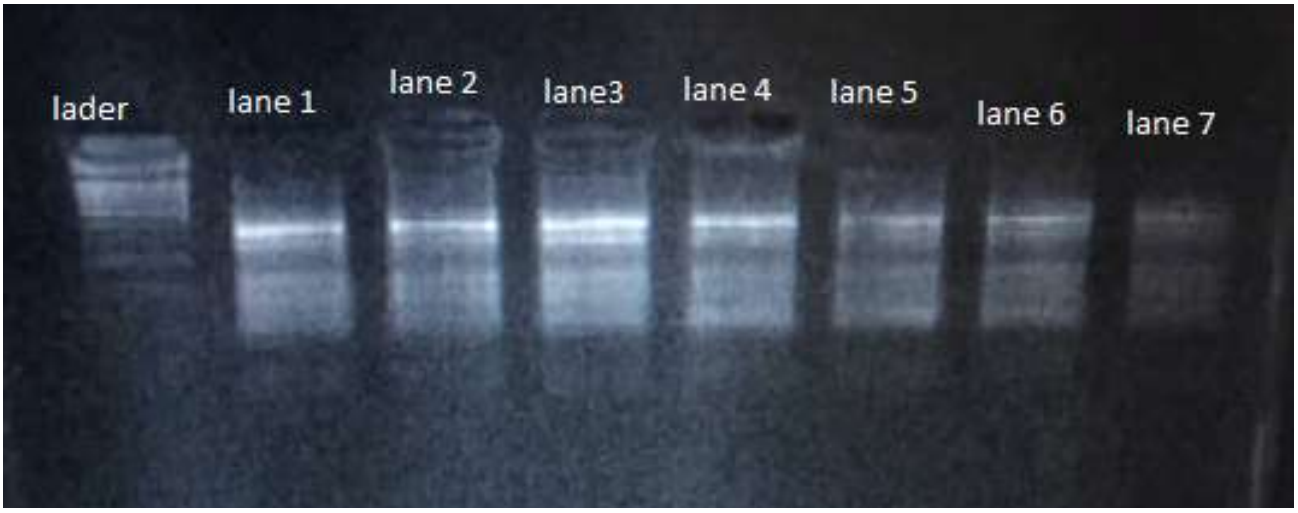

Figure (1) Factor II genewas detected with 345 bp lengthin gel electrophoresis

2.2 Molecular study

In the present study 345bp of *factor II gene* was detected with gel electrophoresis after PCR (Figure 1). The gene was detected in all patients and controls.

2.3 Sequencing:

The sequencing results were analyzed using different bioinformatics soft-wares and tools. The obtained sequences both cases and controls were aligned using BioEdit-ClustalW software with a normal sequence from GenBank gene (accession number NC_000011.10 in NCBI).

Among cases one single Base Exchange was found G to A (G20210A).While no difference between controls and reference sequence was detected. (Figure 2,3)

2.4 Mutation Taster

Mutation taster was used to confirm the mutation which revealed; G>A Base Exchange, disease causing polymorphism was predicted, amino acid sequence was changed, protein features might be affected and splice site also was changed, alteration location was at chromosome 11, alteration type was single base exchange, cDNA changes position was 1825. (Figure 4)

In addition A>G polymorphism was predicted, amino acid sequence was changed, protein features might be affected and splice site also was changed, alteration location was at chromosome 11, alteration type was single base exchange, cDNA changes position was 1792.(figure 5)

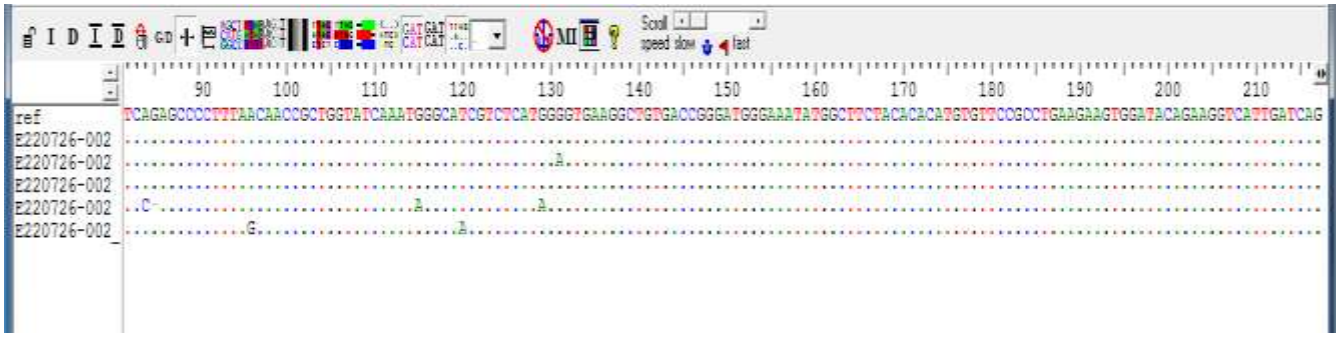

Figure (2): Multiple sequence alignment using Bio-Edit clustal W for cases group with reference gene sequence of II gene.

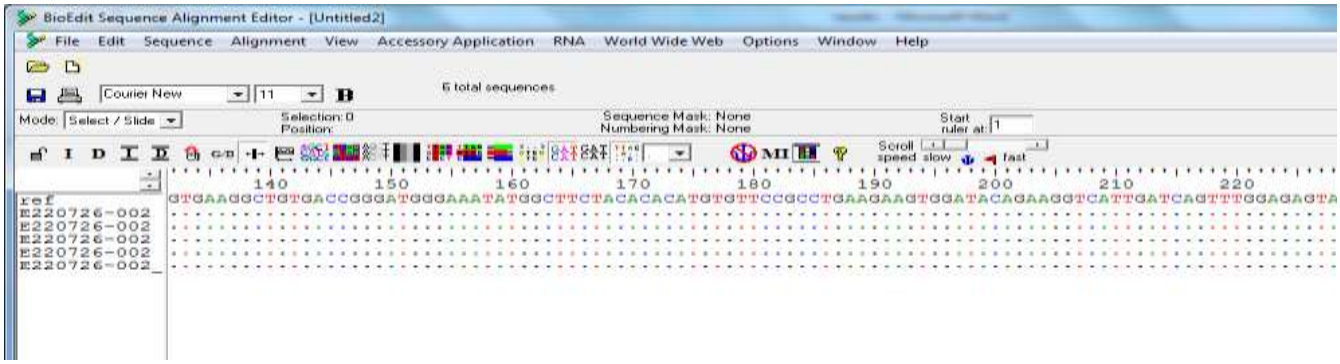

Figure (3): Multiple sequence alignment using Bio-Edit clustal W for control group with reference gene sequence of II gene.

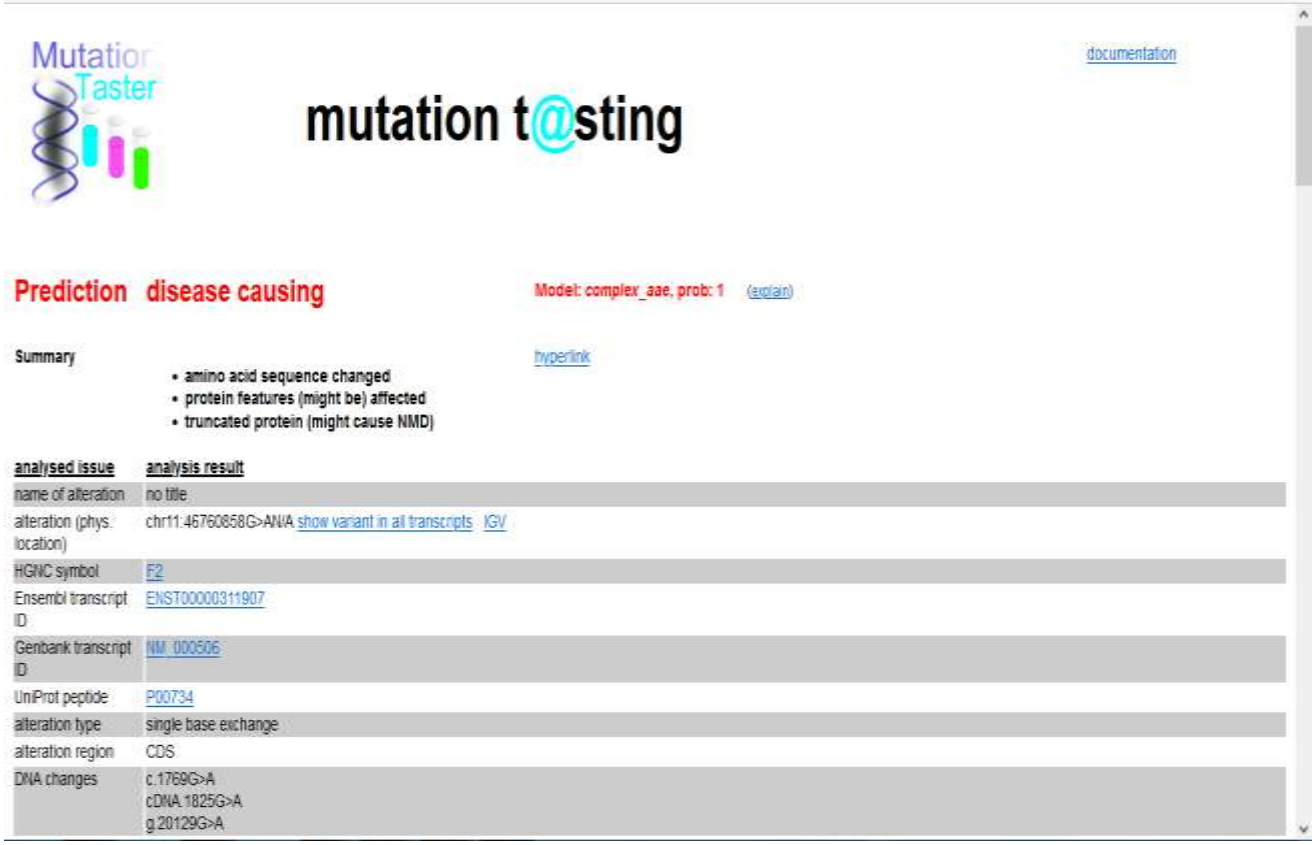

Figure (4): result of G>A singles Base Exchange tested in mutation taster application.

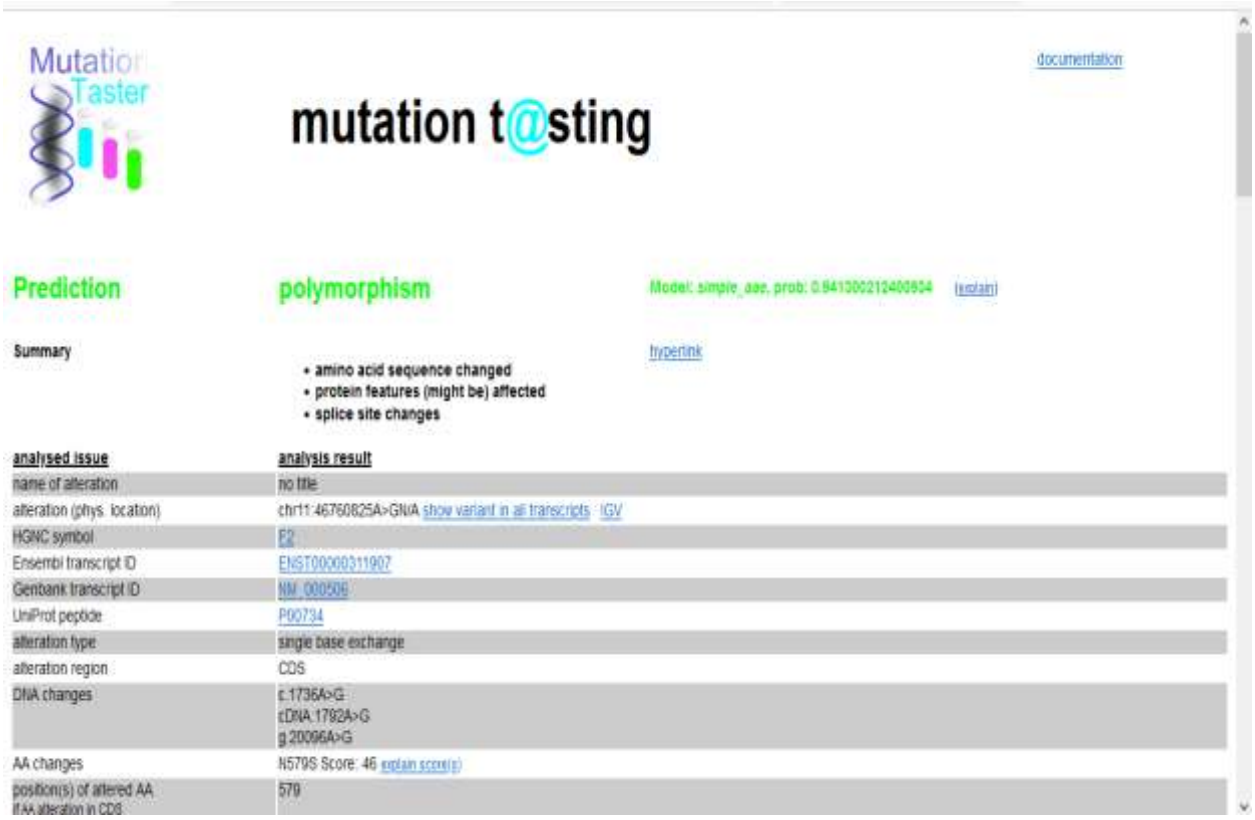

Figure (5): result of A>G singles Base Exchange tested in mutation taster application

Discussion:

Although intracerebral hemorrhage is the third most common cause of cerebrovascular disease, there aren't many hereditary risk factors linked to its occurrence. The risk for thrombosis has recently been linked to several polymorphisms that influence clotting factors. Reports, however, have not properly examined how polymorphisms affect the haemostatic state in bleeding diseases. [6]

Less is known about ICH, despite the fact that the impact of gender on a number of acute brain traumas (including ischemic stroke, traumatic brain injury, and spinal cord injury) has been extensively established. Males are twice as likely as females to have ICH in the current study.

Additionally, ICH is a major cause of death and disability, particularly in young adults (15–45 years old), according to one study on stroke in Africa. Another study looked at how the incidence of ICH rises with advancing age. [7] Additionally, intracerebral hemorrhage is twice as common as subarachnoid hemorrhage, according to Broderick J, Brott T, Tomsick T, Miller R, and Huster G. Our findings regarding age-related issues in J Neurosurg. 1993;78:188–191 showed that age cannot be regarded as a risk factor in the development of ICH. [8]

PT was discovered to be growing throughout hypertension, and while the prevalence of hypertension rises with age, the risk for stroke from hypertension varies. Atherogenic dyslipidaemia, hypertension, glucose intolerance, and a prothrombotic state are frequent metabolic abnormalities in type 2 diabetic individuals. And to add to this, persons with metabolic syndrome have a pattern of coagulation factors that promote thrombosis or inhibit thrombolysis. This prothrombotic condition is a more recently discovered component of the metabolic syndrome. [9,10,11]

Our findings in the present study showed a correlation between ICH and elevated blood pressure and type 2 diabetes, but a large proportion of the patients in this study did not have either of these conditions.

Coagulation factor II (FII), also known as prothrombin, is transformed into its active form, thrombin, in response to vascular injury by prothrombinase, a macromolecular complex made

up of factor Xa (FXa), factor VA (FVA), calcium ions, and phospholipids.[12]

When thrombin enters the bloodstream, it changes fibrinogen into fibrin, activates platelets, and increases endothelial permeability, which stops blood loss at the site of injury and promotes vascular remodeling.[13]

It was discovered that prothrombin-deficient mice die before they should during the embryonic stage as a result of bleeding issues caused by prothrombin, which is encoded by the *F2 gene*. [14]

It was also discovered that the mutation G20210A in the 3′ untranslated region of the *F2 gene* is a well-known risk factor for thrombophilia. Single-nucleotide polymorphisms (SNPs) discovered in patients are frequently associated with mild to severe bleeding phenotypes. [15]

Regarding this gene, Hepatocytes in the liver produce prothrombin as a single pre/pro-polypeptide, indicating that patients with liver disease are more susceptible to bleeding tendencies as a result of this gene deficiency. However, in the current study, our patients do not have liver difficulties.

Additionally, prothrombin undergoes numerous post-translational modifications before being secreted into the plasma. These modifications include removing the pre/pro peptide, which is 3 amino acids long, adding three N-glycosylations at positions 78, 100, and 373, and changing the first 10 residues of glutamic acid (Glu) to -carboxy glutamic acid (Gla). [16,17]

Glycans at position 373 boost the protein's thermodynamic stability and provide proteolysis protection without compromising thrombin's enzymatic activity. [18]

As a result, the severe bleeding disorder is linked to the mutations Glu7 Lys in prothrombin Nijmegen and Glu29 Gly in prothrombin Shanghai.

Bioinformatics software and techniques were used to assess the sequencing results. When the examples were compared to the standard reference, just one Base Exchange—from G to A—was discovered (G20210A). When the controls were compared to the typical reference, no base exchange at all was discovered in any of the control groups.

A mutation taster was used to confirm the mutation, which revealed the following: G>A Base Exchange, disease-causing polymorphism prediction, altered amino acid sequence, potential impact on protein characteristics, and altered splice site.

Factor V Leiden and Prothrombin G20210A, which are brought on by mutations in the genes for prothrombin and factor V, respectively, are the two most prevalent inherited hypercoagulable disorders. Prothrombin G20210A is a mutation in the prothrombin gene's non-coding region that causes elevated protein production (prothrombin levels between 110% and 120%).

The results of this study conflicted with those of BertinaRMetal, who found that the 20210A polymorphism of thrombin was present. In patients with intercerebral hemorrhage, a reduced rate of polymorphism was noted. [3]

In contrast to what Corral et al. observed, our findings in patients with intracranial hemorrhage justify testing for three polymorphisms (20210A, FV Leiden mutation, and VIIDel/Ins2323 polymorphism) that impact the level or function of three important clotting factors: II, V, and VII. Therefore, depending on the presence of specific circumstances and other risk factors for thrombosis or bleeding, one polymorphism giving a specific procoagulant state could have a distinct pathologic effect, raising the risk for thrombosis or reducing that of hemorrhage. [5]

Conclusion:

Factor II gene polymorphism (G20210A) was detected and might be association with intercereberal hemorrhage among Sudanese patients

Abbreviations

ICH : intercereberal hemorrhage
SDH: subdural hemorrhage
EDH: epidural hematoma
IVH: Intraventricular hemorrhage
EVD: External ventricular drains

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Author Contribution

All authors similarly contributed to this manuscript, covered wrote, corrected and authorized this manuscript.

References:

[1] Underwood J.C.E.General and systematic pathology,2004 ;4th (26 :517-518)

[2] Sang Joon An, Tae Jung Kim, Byung-Woo Yoon, Epidemiology, Risk Factors, and Clinical Features of Intracerebral Hemorrhage Journal of Stroke 2017;19(1):3-10

[3] Bertina RM. The prothrombin 20210G to A variation and thrombosis. Curr Opin Hematol. 1998;5: 339-342

[4] Devan WJ, Falcone GJ, Anderson CD, Jagiella JM, Schmidt H, Hansen BM, et al. Heritability estimates identify a substantial genetic contribution to risk and outcome of intracerebral hemorrhage. Stroke J Cereb Circ. 2013;44:1578–1583

[5] Javier Corral, Juan Antonio Iniesta, Rocio Gonza´lez-Conejero, Marino Villalo´ n, and Vicente Vicente. Polymorphisms of clotting factors modify the risk for primary intracranial hemorrhage. BLOOD 2001; 97(10): 2979-2982

[6] Reaven GM. Role of insulin resistance in human disease.Diabetes.1988;37:1595–1607

[7] Grundy SM. Hypertriglyceridemia, a therogenic dyslipidemia, and the metabolic syndrome.Am J Cardiol.1998;81(4A):18B–25B

[8] Nolan RD, Vinik AI.Pathogenesis of platelet dysfunction in diabetes. In: LeRoith D, Olefsky JM, Taylor SI, editors. Diabetes Mellitus: A Fundamental and Clinical Text. Philadelphia: Lippincott-Raven; 1996. pp. 832–839

[9] Krishnaswamy S. The transition of prothrombin to thrombin.J ThrombHaemost. (2013) 11 (Suppl. 1):265–76. 10.1111/jth.12217

[10] RefAriens RA.Fibrin(ogen) and thrombotic disease. J ThrombHaemost. (2013) 11 (Suppl 1):294–305. 10.1111/jth.12229

[11] ref Sun WY, Witte DP, Degen JL, Colbert MC, Burkart MC, Holmback K, et al.. Prothrombin deficiency results in embryonic and neonatal lethality in mice. ProcNatlAcadSci U A. (1998) 95:7597–602

[12] Ref Lancellotti S, Basso M, De Cristofaro R. Congenital prothrombin deficiency: an update. SeminThrombHemost.(2013) 39:596–606. 10.1055/s-0033-1348948

[13] Mann KG, Elion J, Butkowski RJ, Downing M, Nesheim ME. Prothrombin.Methods Enzymol. (1981) 80 (Pt C):286–302

[14] Huang M, Rigby AC, Morelli X, Grant MA, Huang G, Furie B, et al..Structural basis of membrane binding by Gla domains of vitamin K-dependent proteins.Nat Struct Biol. (2003) 10:751–6. 10.1038/nsb971

[15] Frasson R, Pozzi N, Johnson DJD, Huntington JA, De Fillips V. Effect of glycosylation on thrombin structure, stability and function. J ThrombHaemost. (2009) 7:230

[16] Poudel S, Zeb M, Kondapaneni V, Gutlapalli SD, Choudhari J, Sodiya OT, Toulassi IA, Cancarevic I. Association of G20210A Prothrombin Gene Mutation and Cerebral Ischemic Stroke in Young Patients. Cureus. 2020 Dec 8;12(12):e11984. doi: 10.7759/cureus.11984. PMID: 33437541; PMCID: PMC7793372.

[17] Crespin, M.; Alhenc-Gelas, M. Fetal intracerebral hemorrhage in familial thrombophilia. Pediatr. Neurol. **2009**, 41, 291–293.

[18] Javier Corral, Juan Antonio Iniesta, RocioGonza´lez-Conejero, Marino Villalo´ n, and Vicente Vicente. Polymorphisms of clotting factors modify the risk for primary intracranial hemorrhage. BLOOD 2001; 97(10): 2979-2982