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Association of HindIII SNP in Intron 19 of the FVIII gene with musculoskeletal complications in pediatric patients with hemophilia A

Maria Christina Shanty Larasati[1,2], Anang Endaryanto[1,2*], David Nugraha[3], Sukmawati Basuki[3,4], Fedik Abdul Rantam[5], I Dewa Gede Ugrasena[1,2]

[1]Departement of Child Health, Dr. Soetomo General Academic Hospital,Surabaya, Indonesia

[2]Departement of Child Health, Faculty of Medicine- Universitas Airlangga, Surabaya, Indonesia

[3]Malaria Study Group Laboratory of Malaria, Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia

[4]Department of Medical Microbiology and Parasitology, Faculty of Medicine – Universitas Airlangga, Surabaya, Indonesia

[5]Research center for vaccine technology and development (RCVTD), Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia

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ABSTRACT

Hemophilia A (HA) is the most common congenital X-linked coagulopathy caused by mutations in the FVIII gene, inherited in a recessive manner. Despite its well-established clinical characteristics, approximately 30% of HA cases are sporadic. Musculoskeletal complications, such as recurrent joint bleedings, significantly impact patient quality of life. This study aimed to investigate the relationship between the *Hind*III polymorphism in the FVIII gene and musculoskeletal complications, as well as its association with family history in HA patients from East Java, Indonesia. A total of 63 patients were analyzed using the PCR-RFLP method. The findings revealed no significant correlation between the *Hind*III SNP and musculoskeletal complications (P = 0.720) or family history (P = 0.542). These results suggest that the *Hind*III polymorphism does not serve as a determinant factor for musculoskeletal complications or family history in this population. Further studies with larger sample sizes and advanced molecular techniques are needed to explore the role of the *Hind*III polymorphism in hemophilia A.

Keywords: *Hemophilia A, HindIII polymorphism, FVIII gene, musculoskeletal complications, family history, genetic markers, PCR-RFLP.*

1. INTRODUCTION

Haemophilia A (HA) is the most common congenital X-linked coagulopathy caused by mutations in the factor VIII (FVIII) gene, and transmitted genetically according to a recessive mode (Fernandez, 2019; Johnny, 2019). However, about 30% of patients with HA are sporadic cases with a negative family history. This genetic anomaly is characterized by a deficiency of FVIII with an occurrence rate of around 1 in 5000 to 10.000 male persons worldwide (Laffan et al., 2011; Schep et al., 2019). The clinical manifestations associated with haemophilia correspond to uncontrolled internal haemorrhagic episodes. The severity and frequency of these manifestations depend directly on the level of circulating plasma FVIII. The numerous bleeding episodes that individuals with severe haemophilia experience can lead to long-term disability. Recurrent joint bleedings can result in severe arthropathy, muscle atrophy, pseudo-tumours, and lead to chronic pain and impaired mobility that often requires surgery and arthroplasty to improve joint function (Castro et al., 2014; Doncel et al., 2023).

Haemophilia is diagnosed in the laboratory by coagulation test, genetic assessment of haemophilia is important to define disease biology, establish diagnosis in difficult cases, predict risk of inhibitor development, and provide a prenatal diagnosis if desired (Spena et al., 2018; Jerzy et al., 2022). Wherever possible, genotype analysis should be offered to all patients with haemophilia. The mainstay of treatment for haemophilia A involves replacing the missing blood coagulation FVIII when bleeding episodes occur (on-demand treatment) or by scheduled infusions several times per week (prophylaxis treatment). Both plasma-derived and recombinant clotting factor concentrates are suitable for these different strategies of haemophilia management (Franchini & Mannucci, 2016; Mahlangu, 2019; Zhou et al., 2024).

Nowadays, many studies are being carried out on polymorphisms within the genes coding for FVIII (Campos et al, 2018). They have clinical relevance in the context of hereditary disorders in that they can be used to detect a defective (or normal)

patient through an affected family. Linkage studies have been used to study carrier status and prenatal diagnosis in HA. The FVIII gene contains several SNPs, many of which fall into the subcategory of restriction fragment length polymorphisms (RFLPs), such as the alleles of *Hind*III RFLPs. Depending on the nature of the mutation that causes the disease, the affected clotting factor may be completely absent from the patient's body, or present but in a dysfunctional form. Full FVIII gene screening is performed by polymerase chain reaction (PCR) and Sanger sequencing, or next-generation sequencing. The approach and use of a specific technique depend on the available technical expertise and resources. That's why we used the RFLP-PCR method that was available to us. There are no studies regarding polymorphisms *Hind*III of FVIII genes and its relationship with joint bleedings complaints in Indonesia especially East Java.

2. METHODS

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Research location

The study was conducted at the Dr. Soetomo General Hospital which has a unit for the care of haemophilia patients in Surabaya East Java

Enrolment Procedure, Sample Collection and DNA Extraction

Oral, written and informed consent was obtained prior to any sampling from the interviewees. Indeed, before obtaining this consent, the interviewees were informed of the objectives of the study, explaining that the information collected would be strictly anonymous and confidential. They were also informed of the guarantees taken with regard to the security of the information collected. Before obtaining their consent (signature), parents of under-age patients were truly informed about the nature of the study, the risks and benefits, as well as the possibility of interrupting their participation or that of their child in the study at any time. In order to guarantee the confidentiality of the information collected, all patients selected for the study were registered with an identification code. Moreover, this study received approvals of the Ethics Committee of the Dr. Soetomo General Hospital. A total of 63 patients were enrolled in the study. These were all collected after completing a questionnaire on medical record information, 3 mL of blood was collected by venipuncture and aliquoted into ethylenediaminetetraacetic acid (EDTA) tubes and submitted to Institute Tropical Diseases Universitas Airlangga at Surabaya.

Plasma was obtained from EDTA-anticoagulated whole blood by centrifugation and stored at -20°C.

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)

This PCR-RFLP method has been widely used for detecting genetic polymorphisms and is considered a reliable approach for identifying variations in the FVIII gene (Sambrook & Russell, 2001; Kumar et al., 2019). The *Hind*III polymorphism of the FVIII gene was analyzed using a modified PCR-RFLP protocol. The primer sequences utilized for FVIII gene amplification were FVIII-S (5'GTGAGTAGCAGTGTGGGCAGA 3') and FVIII-A (5'CTGAAATGAAACGGGTGGAAC 3'), which were designed to target the specific polymorphic site. The polymerase chain reaction (PCR) was conducted in a 10 µL reaction mixture, which included standard PCR buffer, 0.25 µL $MgCl_2$, 2.25 µL H_2O, 2 µL DNA sample, and 5 µL GoTaq DNA polymerase. The thermal cycling conditions involved an initial denaturation at 95°C for 5 minutes, followed by 35 cycles consisting of denaturation at 95°C, annealing at 62°C for 55 seconds, and elongation at 72°C for 1 minute, with a final extension at 72°C for 5 minutes.

To identify the *Hind*III polymorphism, 5 µL of the PCR product was incubated with the *Hind*III restriction enzyme at 37°C for 3 hours, followed by enzyme inactivation at 80°C for 30 minutes, and a final cooling step at 4°C for 1 minute. The digested DNA fragments (608 bp) were separated using an 8% acrylamide gel and visualized through electrophoresis, which was performed in a 1× TBE buffer chamber at 200 Amp for 20 minutes or 110 V for 50 minutes.

Analysis Data

The study focused on the *Hind*III polymorphism of FVIII and the occurrence of joint bleeding in patients with hemophilia. Additionally, family history and age were examined. The data collected was qualitative, and a descriptive analysis was performed to explore potential associations between the *Hind*III polymorphism of FVIII and musculoskeletal complications. Statistical analysis was conducted using STATA 15 software, with a significance level set at 5%. A p-value of 0.05 or lower was considered statistically significant.

3. RESULTS & DISCUSSION

Single nucleotide polymorphism (SNP) *Hind*III in the factor VIII (FVIII) gene is a genetic variation in intron 19 of the FVIII gene. SNPs are commonly used as genetic markers in hemophilia A studies, as they can identify allelic variations through restriction fragment length polymorphism (RFLP) analysis. This method utilizes the *Hind*III restriction enzyme, which recognizes and cuts specific DNA sequences, resulting in a cutting pattern that can be used to differentiate between different alleles (Abdulqader et al., 2019; Doncel et al., 2023).

Population Characteristics

The results of this study indicate that the majority of hemophilia patients analyzed are within the age range of 5-18 years (77.8%), with fewer cases in the 1-4 years age group (20.6%) and less than 1 year (1.6%). This is consistent with previous studies which revealed that the clinical manifestations of hemophilia are more frequently diagnosed in children, especially when they begin to be active and experience minor trauma that can trigger bleeding (Soucie et al., 2018; Singh et al., 2020; Vantaku et al., 2023). Analysis of family history showed that 55.6% of patients have a family history of hemophilia, while 44.4% do not. These findings support the fact that hemophilia is a genetic disorder with an X-linked recessive inheritance pattern, more commonly found in males with the mother being the carrier of the genetic mutation (Biguzii et al., 2019; Srivastava et al., 2020). Data on musculoskeletal complications were found in nearly half of the patients (49.2%), while 50.8% did not experience complications. Musculoskeletal complications, such as recurrent hemarthrosis and hemophilic arthropathy, are common long-term consequences in hemophilia patients, especially those with limited access to prophylactic therapy (Rodriguez-Merchan, 2010; Lobet et al., 2021). From the analysis of SNP *Hind*III polymorphism in the FVIII gene, it was found that 61.9% of patients had the wild type, while 38.1% had variants other than the wild type. This polymorphism has been associated with varying levels of factor VIII expression and affects the severity of the disease as well as musculoskeletal complications in hemophilia patients (Goodeve, 2015). It can be concluded that the population data highlights the importance of early monitoring of genetic factors and family history in assessing the risk of complications in hemophilia patients. Early detection and proper management can help in preventing further complications, particularly those related to the musculoskeletal system (Srivastava et al., 2020; WFH, 2020).

Table 1. Patient characteristics of the study population

Patient characteristics	Number of case	Frequency (%)
Age		
≤ 1 years	1	1.6
> 1 – 4 years	13	20.6
5 – 18 years	49	77.8
Family history of hemophilia		
Yes	35	55.6
No	28	44.4
Musculoskeletal complications		
Yes	31	49.2
No	32	50.8
SNP HindIII gen FVIII		
Wild type	39	61.9
Mutant	24	38.1

The relationship between *Hind*III and musculoskeletal complications and family history

The study investigating the association between the *Hind*III polymorphism of the FVIII gene and a family history of hemophilia demonstrated no significant relationship (P = 0.542). Our findings indicate that both individuals with and without a family history of hemophilia exhibited the *Hind*III polymorphism of the FVIII gene at similar proportions. Moreover, our study revealed no significant association between the *Hind*III polymorphism of the FVIII gene and musculoskeletal complications in hemophilia (P = 0.720) (Table 2). The presence of the *Hind*III polymorphism did not appear to influence the occurrence of musculoskeletal complications among the study participants. These results suggest that the *Hind*III polymorphism of the FVIII gene is not a determinant factor for either a family history of hemophilia or musculoskeletal complications in hemophilic patients.

Table 2. Relationship of *Hind*III SNP of FVIII gene with family history and musculoskeletal complications of

hemophilia

***SNP HindIII* gen FVIII**	**Family History With Hemophilia**				**P**
	Yes		**No**		
	N	**%**	**N**	**%**	
Wild type	20	51.3	19	48.7	0.542
Mutant	15	62.5	9	37.5	

***SNP HindIII* gen FVIII**	**Musculoskeletal Complications**				**P**
	Yes		**No**		
	N	**%**	**N**	**%**	
Wild type	18	46.2	21	53.8	0.720
Mutant	13	54.2	11	45.8	

Our research findings align with several studies regarding the clinical significance evaluation of the *Hind*III SNP in hemophilia A. For example, the study by Ramezani et al. (2016) found that the *Hind*III SNP does not have a direct relationship with the severity of hemophilia, but it can serve as a marker in haplotype analysis to detect carriers of hemophilia A in specific populations. Another study by Bhattacharya et al. (2019) confirmed that the *Hind*III SNP, along with other polymorphisms, can be used as a diagnostic tool to identify carriers of hemophilia A in families with a history of the disease. Although the *Hind*III SNP is located in a non-coding intronic region, some studies suggest that intronic SNPs may affect gene expression through their influence on splicing processes or mRNA stability. A study by Campos et al. (2018) found that certain intronic SNPs in the FVIII gene can affect FVIII plasma activity, although the specific impact of the *Hind*III SNP is not yet fully understood. Thus, while the *Hind*III SNP is a genetic variation commonly used as a marker in hemophilia A studies, no significant relationship with family history or musculoskeletal complications was found in this study. Further research with larger sample sizes and more in-depth molecular analysis is needed to better understand the role of the *Hind*III SNP in hemophilia A.

4. CONCLUSION

In this study, the *Hind*III polymorphism in the FVIII gene was investigated for its potential association with musculoskeletal complications and family history in hemophilia A patients. The results showed no significant correlation between the *Hind*III SNP and the occurrence of musculoskeletal complications or family history of hemophilia. Although this polymorphism is commonly used as a genetic marker in hemophilia A studies, it does not appear to be a determinant factor for these clinical aspects in the studied population. These findings suggest that while the *Hind*III polymorphism may be useful for identifying genetic variations, further research with larger sample sizes and more detailed molecular analyses is necessary to better understand its role in the severity and complications of hemophilia A.

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