

Association of Intron 22 inversion and HLA-DRB1*15:01 allele with inhibitor development in hemophilia A: A genetic analysis of risk factors

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ABSTRACT

Background: Inhibitor development in hemophilia A is a major clinical challenge, leading to severe bleeding complications and reduced quality of life. This multifactorial process is influenced by genetic, immunological, and treatment-related factors.

Objectives: To determine the association between intron 22 inversion (Inv22) and the HLA-DRB1*15:01 allele with inhibitor development.

Materials and methods: This study investigated inhibitor development during initial and subsequent treatments, assessing the association between Inv22 and HLA-DRB1*15:01 with inhibitor formation in 40 hemophilia A patients, 40 non-hemophilia A patients, and 40 healthy controls.

Results: The study found no significant association between Inv22 or HLA-DRB1*15:01 allele and inhibitor development during initial (odds ratio (OR): 0.60, 95% CI: 0.11-3.43, $p=0.697$) or subsequent treatments (OR: 1.33, 95% CI: 0.30-5.93, $p=1.000$). Similarly, HLA-DRB1*15:01 allele showed no significant correlation (initial: OR: 0.25, 95% CI: 0.06-1.13, $p=0.089$; subsequent: OR: 0.82, 95% CI: 0.24-2.84, $p=1.000$). However, patients with both genetic factors had a significantly higher risk of developing inhibitors (OR: 7.90, 95% CI: 1.88-33.06, $p=0.007$).

Conclusion: This study found no significant association between Inv22 or the HLA-DRB1*15:01 allele and inhibitor development in hemophilia A patients during initial or subsequent treatments. However, their combined presence significantly increased the risk of inhibitor formation, suggesting a potential interactive effect requiring further investigation.

Introduction

Development of Factor VIII (FVIII) inhibitors represents the most formidable complication in Hemophilia A management today. While therapeutic advancements have significantly reduced mortality, the emergence of neutralizing antibodies—affecting approximately 25-30%^{1,2} of patients globally and up to 17% of severe cases in Thailand³—renders standard replacement therapy ineffective. This failure of immune tolerance not only escalates the risk of severe bleeding and disability but also necessitates costly bypassing agents,¹ thereby imposing a profound clinical and economic burden. The etiology of inhibitor formation is

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multifactorial, driven by a complex interplay between environmental exposures and genetic predisposition.⁴ Central to this pathogenesis is the immune recognition of therapeutic FVIII as a neo-antigen, a process heavily influenced by the nature of *F8* gene mutations and the host's Major Histocompatibility Complex (MHC) profile.^{4,5} Intron 22 inversion (*Inv22*), responsible for nearly half of severe Hemophilia A cases, causes a complete disruption of FVIII synthesis. In 2019, at Ramathibodi Hospital, the prevalence of *Inv22* among patients with severe hemophilia A was 35.6%.³ However, its independent role as a predictor for inhibitor development remains controversial, with prior studies yielding conflicting associations.⁴ Concurrently, Class II Human Leukocyte Antigens (HLA), specifically the HLA-DRB1*15:01 allele, are implicated in efficient peptide presentation to T-cells. While HLA-DRB1*15:01 is a globally recognized risk factor,⁵ local evidence indicates it is the only allele significantly associated with inhibitors in Thai patients (19.4% vs. 5.0% in controls, $p=0.021$),⁶ exceeding the 8.09% background frequency observed in the general Thai population.⁷ whereas alleles like DRB1*14 and DRB1*07 show no such significance.⁶ These inconsistencies are frequently attributed to variations in sample sizes, study designs, and the diverse genetic backgrounds of different ethnic cohorts.⁵ Yet, when analyzed in isolation, HLA-DRB1*15:01 often appears as a weak risk factor, suggesting that the mechanisms driving immunogenicity are likely synergistic rather than singular.⁸ The synergy likely arises because *Inv22* creates a 'null' state where FVIII is perceived as entirely foreign, which HLA-DRB1*15:01 then efficiently presents to the immune system.

This discrepancy in current literature highlights a critical gap in understanding how structural genetic defects interact with immune response genes. To advance the frontier of precision medicine, this study aims to elucidate the association and potential interaction between *Inv22* and the HLA-DRB1*15:01 allele in the context of inhibitor development within the Thai population. For instance, identifying such high-risk genetic profiles can guide personalized prophylaxis or the early use of non-factor therapies like emicizumab to prevent inhibitor formation.⁹

Unraveling these genetic complexities is essential for constructing robust risk stratification models, ultimately enabling clinicians to personalize therapeutic strategies and optimize outcomes for high-risk patients.

Materials and methods

Participants and sample collection

This study included 120 participants, consisting of 40 hemophilia A patients (FVIII activity (FVIII:C) <50%), 40 non-hemophilia A patients (FVIII:C ≥50%), and 40 healthy individuals as controls. Among hemophilia A patients, the severity was classified into severe (N=27, 67.5%; FVIII:C<1%), moderate (N=10, 25.0%; FVIII:C

1-5%), and mild (N=3, 7.5%; FVIII:C >5%). Participant data included gender, age, FVIII activity (initial and post-treatment), age at first treatment, post-treatment inhibitor levels (Bethesda units; BU), coagulation test (aPTT), treatment type, and 2 mL collected EDTA blood samples. All samples were collected from Maharat Nakhon Ratchasima Hospital, Thailand, during treatment and follow-up. Inhibitor titer was defined by the Bethesda unit (BU),¹⁰ representing the amount of inhibitor that neutralizes 50% of FVIII activity after 2-hour incubation at 37 °C with normal plasma; a titer 0.6 BU was considered positive. Inclusion criteria for "Non-hemophilia A" required a confirmed FVIII:C>50%, while "Healthy individuals" showed no coagulation test abnormalities. Exclusion criteria for all groups included individuals who had not received treatment for over one year. To reduce comparison bias, control groups were randomly selected without matching.

Genotyping of the *Inv22* and HLA-DRB1*15:01 allele

Genomic DNA was extracted from EDTA-anticoagulated whole blood using the Wizard® Genomic DNA Purification Kit (Promega, USA) according to the manufacturer's instructions. Intron 22 inversion was genotyped using the inverse shifting (IS)-PCR method, modified from Rossetti *et al.*¹¹ Briefly, 20 µL of genomic DNA (100 ng/µL) was digested with 20 U of *Bcl*I restriction enzyme (Thermo Fisher Scientific, USA) at 55 °C for 4 hours in a 50 µL reaction volume. The reaction was heat-inactivated at 80 °C for 30 minutes. Circularization of the digested DNA was performed using 3 U of T4 DNA ligase (Thermo Fisher Scientific, USA) in a 400 µL reaction volume at 4 °C overnight. Ligated DNA was purified using the QIAquick® PCR & Gel Cleanup Kit (QIAGEN, Germany) and eluted in 20 µL of distilled water. Amplification was performed using the IS-PCR method in a 20 µL reaction containing 4 µL of circularized DNA, 2x Green PCR Master Mix (Biotech rabbit, Germany), and 0.5 µM of each primer (ID: 5'-ACATACGGTTTAGTCACAAGT-3', IU: 5'-CCTTCAACTCCATCTCCAT-3', 2U: 5'-ACGTGTCTTTGGAGAAGTC-3', 3U: 5'-CTCACATTGTGTTCTGTAGTC-3'). PCR cycling conditions consisted of 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 1 minute, and extension at 72 °C for 1 minute. Results were interpreted by band size: 487-bp for wild-type and 333-bp for *Inv22*.

For detection of the HLA-DRB1*15:01 allele, genomic DNA was amplified using the PCR-SSP method, as previously described by Delfan *et al.*¹² PCR was carried out in a final volume of 20 µL containing 100 ng of genomic DNA, 2x Green PCR Master Mix (Biotech rabbit, Germany), and 0.5 µM of each primer. The HLA-DRB1*15:01-specific primers used were forward 5'-TCCTGTGGCAGCCTAAGAG-3' and reverse 5'-CCGCGCCTGCTCCAGGAT-3'. The human growth hormone (HGH) gene served as an internal control, with forward primer 5'-TGCCTTCCCAACCATTCCTTA-3' and reverse primer 5'-CCACT

CACGGATTCTGTTGTGTTTC-3'. PCR amplification was performed for 35 cycles under the following conditions: denaturation at 95°C for 30 seconds, annealing at 60°C for 45 seconds, and extension at 72 °C for 30 seconds. All PCR products were separated on a 1.5% agarose gel and visualized under UV illumination. The 197-bp and 434-bp bands represented the HLA-DRB1*15:01 allele and the HGH internal control, respectively.

Association analysis of *Inv22* and HLA-DRB1*15:01 allele influence on inhibitor development in hemophilia A

The Bethesda assay was used to measure inhibitors in hemophilia A patients, with levels quantified in BU. An inhibitor was defined as a level exceeding 0.6 BU.¹⁰ The presence of the *Inv22* and HLA-DRB1*15:01 allele was analyzed in relation to inhibitor formation at two time points: the first occurrence of an inhibitor (first inhibitor) and at any point during treatment. The relationship between *Inv22*, HLA-DRB1*15:01 allele, and inhibitor production in hemophilia A patients was tested using Fisher's exact test or Chi-squared test, with odds ratios (OR) and 95% confidence intervals (CI) calculated to quantify the strength of these associations. The Chi-squared test was used primarily, while Fisher's exact test was selected when any expected cell count was less than 5. This study

also demonstrates the relationship between genetic determinants and inhibitor development following initial treatment and at any point during treatment. A $p < 0.05$ indicated a statistically significant association between genetic mutations and inhibitor production. Statistical analyses were performed using IBM SPSS Statistics version 26 (SPSS Inc., Chicago, IL, USA).

Results

Participants data

A descriptive analysis was performed on gender, aPTT test, FVIII activity (initial and post-treatment), inhibitor level (BU), and treatment type (Table 1). The study enrolled 120 subjects: 40 Hemophilia A (HA) patients, 40 non-HA patients, and 40 healthy controls. The HA cohort was exclusively male. As anticipated, HA patients exhibited profound coagulopathy, characterized by markedly prolonged aPTT (>82 s) and severe FVIII deficiency (<5.2%) compared to controls. All HA patients received FVIII concentrate therapy. Notably, 30% (12/40) of HA patients presented with inhibitors initially, with the prevalence rising to 50% (20/40) cumulatively during treatment. Inhibitor titers were clinically substantial in positive cases (mean 16.12±21.57 BU), contrasting sharply with the negligible levels observed in non-inhibitor patients.

Table 1. Descriptive analysis of gender, age, FVIII Activity (%), inhibitor levels, and treatment among hemophilia A (inhibitor and non-inhibitor during initial or subsequent treatment), non-hemophilia A, and normal control groups.

Factors	Hemophilia A patients (First inhibitor) (N=40)		Hemophilia A patients (Inhibitors at any point during treatment) (N=40)		Non-hemophilia A (%) (N=40)	Normal (%) (N=40)
	Inhibitor (%)	Non-inhibitor (%)	Inhibitor (%)	Non-inhibitor (%)		
Number (%)	12 (30)	28 (70)	20 (50)	20 (50)	40 (100)	40 (100)
Sex						
- Male (%)	12 (100)	28 (100)	20 (100)	20 (100)	24 (60)	21 (52.5)
- Female (%)	0 (0)	0 (0)	0 (0)	0 (0)	16 (40)	19 (47.5)
Age (mean±SD)	23.54±10.51	14.26±12.33	18.68±10.67	15.21±14.08	33.60±25.05	62.5±17.70
Range	6-35	1-44	6-35	1-44	3-82	22-91
aPTT test (sec) (Mean±SD)	86.1±19.9	82.5±21.9	84.5±16.7	82.7±25.3	33.9±12.6	26.1±2.5
% FVIII activity (first time visiting; Mean±SD)	4.38±11.24	4.03±7.21	3.17±8.71	5.11±8.33	194.57±80.66	Not test
% FVIII activity (post-treatment; Mean±SD)	31.09±41.70	8.01±17.94	17.19±35.10	10.96±20.61	Not test	Not test
Inhibitor level (BU; Mean±SD)	16.12±21.57	0.41±0.02	9.84±13.21	0.41±0.02	Not test	Not test
Therapy by FVIII concentrate	12 (100)	28 (100)	20 (100)	20 (100)	Not treatment	Not treatment

Genotyping of the *Inv22* and *HLA-DRB1*15:01* allele

The detection of the *Inv22* and *HLA-DRB1*15:01* allele in blood samples was performed using the primers described above. Genotyping for *Inv22* and *HLA-DRB1*15:01* was successfully performed in all participants. Genotypes were determined based on the specific PCR product sizes as described in the Materials and Methods section. The results of this study demonstrated that the PCR product sizes corresponded to the expected genotypes (Figure 1),

with *Inv22* identified in 9 out of 40 hemophilia A patients (22.5%).

For the detection of the *HLA-DRB1*15:01* allele, this study utilized the HGH gene as an internal control. The presence of the *HLA-DRB1*15:01* allele was indicated by the specific PCR product shown in Figure 2. Based on the results, the *HLA-DRB1*15:01* allele was detected in 19 of 40 hemophilia A patients (47.5%), 17 of 40 non-hemophilia A individuals (42.5%), and 12 of 40 healthy controls (30.0%).

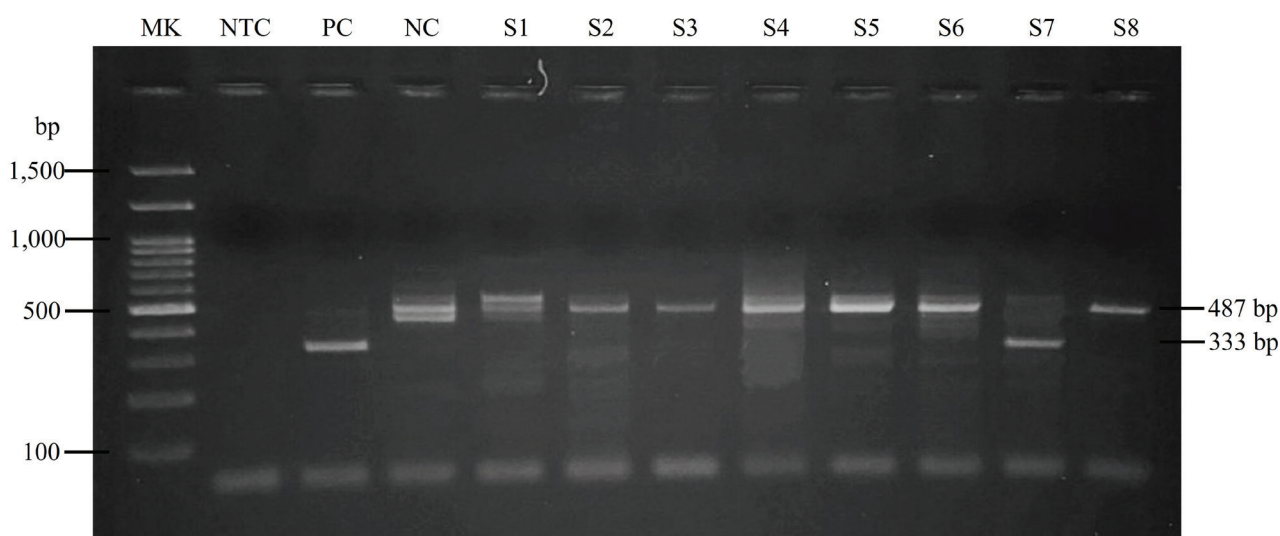


Figure 1. The genotyping of *Inv22* gene in hemophilia A patients using the IS-PCR method. MK: 100 bp DNA ladder, NTC: non-template control, PC: positive control containing the *Inv22* gene, NC: DNA sample from a hemophilia A patient negative for the *Inv22* gene, Lanes S1-S8: samples from hemophilia A patients 1 through 8.

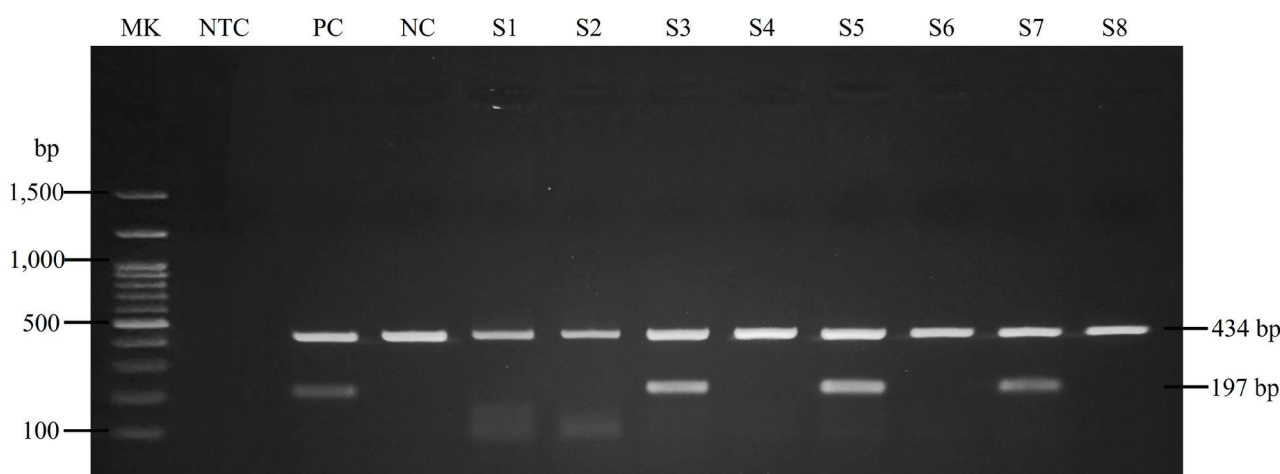


Figure 2. The genotyping of *HLA-DRB1*15:01* allele using the PCR-SSP method in a cohort of 120 individuals. MK: 100 bp DNA ladder, NTC: non-template control, PC: positive control containing the *HLA-DRB1*15:01* allele, NC: DNA sample from an individual negative for the *HLA-DRB1*15:01* allele, Lanes S1-S8 : samples from individuals 1 through 8.

Association analysis of *Inv22* and *HLA-DRB1*15:01* allele influence on inhibitor development in hemophilia A

The genetic analysis of study groups in relation to first-inhibitor development in hemophilia A patients identified *Inv22* in 7.5% (N=9) of participants, exclusively among those with hemophilia A (Table 2). It was present in 17% of inhibitor-positive and 25% of non-inhibitor patients, while absent in non-hemophilia A and normal individuals. The odds ratio for *Inv22* in inhibitor-positive hemophilia A patients was 0.60 (95% CI: 0.11-3.43, $p=0.697$), indicating no significant association with inhibitor development.

For the *HLA-DRB1*15:01* allele, 40% (N=48) of participants were positive (Table 2). It was found in 25% of inhibitor-positive and 57% of non-inhibitor hemophilia A patients, compared to 43% of non-hemophilia A and 30% of normal individuals. The odds ratio for *HLA-DRB1*15:01* allele positivity in inhibitor-positive hemophilia A patients was 0.25 (95% CI: 0.06-1.13, $p=0.089$). No significant association was observed between this genetic marker and inhibitor formation ($p>0.05$). This result indicates that the presence of this genotype did not significantly influence the risk of inhibitor development in the studied population.

Table 2. Genetic analysis of the *Inv22* and the *HLA-DRB1*15:01* allele and their association with initial inhibitor development in hemophilia A patient's post-treatment.

Allele	Hemophilia A (N=40)		Non-Hemophilia A (N=40)	Normal (N=40)	Total (N=120)
	Inhibitor (N=12)	Non-inhibitor (N=28)			
<i>Inv22</i> inversion positive (%)	2 (17)	7 (25)	0	0	9 (7.5)
<i>Inv22</i> inversion negative (%)	10 (83)	21 (75)	40 (100)	40 (100)	111 (92.5)
Odd ratio (95% CI)	0.60 (0.11-3.43)		19.29 (0.86-432.99)	19.29 (0.86-432.99)	
<i>p</i> value	0.697		0.062	0.062	
<i>HLA-DRB1*15:01</i> positive (%)	3 (25)	16 (57)	17 (43)	12 (30)	48 (40)
<i>HLA-DRB1*15:01</i> negative (%)	9 (75)	12 (43)	23 (57)	28 (70)	72 (60)
Odd ratio (95% CI)	0.25 (0.06-1.13)		0.45 (0.11-1.92)	0.78 (0.18-3.39)	
<i>p</i> value	0.089		0.330	1.000	

Genetic analysis based on inhibitor development at any treatment stage showed that *Inv22* occurred in 7.5% (N=9) of participants, exclusively among hemophilia A patients (Table 3). Within this group, 25% of inhibitor-positive and 20% of non-inhibitor patients carried the inversion, whereas all non-hemophilia A and normal individuals were negative. The odds ratio for *Inv22* in inhibitor-positive hemophilia A patients was 1.33 (95% CI: 0.30-5.93, $p=1.00$), indicating no significant association with inhibitor development. However, a significant difference was observed between hemophilia A and non-hemophilia A groups

($p=0.026$), suggesting a strong link between *Inv22* and hemophilia A. For the *HLA-DRB1*15:01* allele, 40% (N=48) of participants tested positive. Among hemophilia A patients, 45% of inhibitor-positive and 50% of non-inhibitor individuals carried this allele, compared to 43% of non-hemophilia A and 30% of normal individuals. The odds ratio for *HLA-DRB1*15:01* allele positivity in inhibitor-positive hemophilia A patients was 0.82 (95% CI: 0.24-2.84, $p=1.00$), showing no significant association with inhibitor development. Similarly, no statistically significant differences were found between non-hemophilia A and normal groups.

Table 3. Genetic analysis of the Inv22 and the HLA-DRB1*15:01 allele and their association with the development of inhibitors in hemophilia A patient at any point during treatment.

Allele	Hemophilia A (N=40)		Non-Hemophilia A (N=40)	Normal (N=40)	Total (N=120)
	Inhibitor (N=20)	Non-inhibitor (N=20)			
Inv22 inversion positive (%)	5 (25)	4 (20)	0	0	9 (7.5)
Inv22 inversion negative (%)	15 (75)	16 (80)	40 (100)	40 (100)	
Odd ratio (95% CI)	1.33 (0.30-5.93)		28.74 (1.50-551.18)	28.74 (1.50-551.18)	111 (92.5)
p value	1.000		0.026*	0.026*	
HLA-DRB1*15:01 positive (%)	9 (45)	10 (50)	17 (43)	12 (30)	48 (40)
HLA DRB1*15:01 negative (%)	11 (55)	10 (50)	23 (57)	28 (70)	72 (60)
Odd ratio (95% CI)	0.82 (0.24-2.84)		1.11 (0.38-3.27)	1.91 (0.63-5.80)	
p value	1.000		1.000	0.268	

*Statistically significant

Genetic analysis of Inv22 and HLA-DRB1*15:01 allele in the study population showed an odds ratio (OR) of 3.34 (95% CI: 0.59-18.88) for the first inhibitor occurrence, though this association was not statistically significant ($p=0.407$) (Table 4). However, for inhibitors developed at any point during treatment, both intron 22

inversion and HLA-DRB1*15:01 allele were significantly associated with an increased risk, with an OR of 7.90 (95% CI: 1.88-33.06, $p=0.007$). These findings suggest that these genetic factors may influence inhibitor formation over the course of treatment rather than at the initial occurrence.

Table 4. Association of Inv22 and HLA-DRB1*15:01 allele with first-time and any-time inhibitor development in hemophilia A patients.

Inhibitor	Allele	OR (95%CI)	p value
First inhibitor	Inv22+HLA-DRB1*15:01 allele	3.34 (0.59-18.88)	0.407
Inhibitor at any-time inhibitor development	Inv22+HLA-DRB1*15:01 allele	7.90 (1.88-33.06)	0.007*

* Statistically significant

Discussion

Genetic factors play a significant role in inhibitor development among hemophilia A patients, particularly the type of mutation in the FVIII gene and variations in HLA class II alleles.⁴ Severe, null mutations such as Inv22, large deletions, and nonsense mutations can lead to a complete absence of FVIII protein, heightening the likelihood that administered FVIII will be recognized as foreign and elicit an immune response. Conversely, milder mutations (e.g., missense mutations) are less immunogenic, resulting in a lower incidence of inhibitor formation.⁴ In parallel, certain HLA class II alleles, including HLA-DRB1*15:01 allele, have been implicated in the antigen presentation process that underlies inhibitor development.⁵ These alleles may predispose individuals to a more robust T-cell-mediated immune response against FVIII, thus increasing inhibitor risk.¹³ This study analyzed whether the Inv22 and HLA-DRB1*15:01 allele influence inhibitor development in hemophilia A patients. As shown in Table 1, Factor VIII activity was higher in hemophilia A patients with

inhibitors than in those without, regardless of whether inhibitors developed after initial treatment or at any point later. This reflects the treatment strategy of administering high-dose factor VIII concentrate to induce immune tolerance and suppress inhibitor formation.¹³ This study found the frequency of the Inv22 to be 22.5% (9 of 40 hemophilia A patients), which is slightly lower than the 30-50% reported in previous studies.¹⁴ Many other studies in Thailand found that the allele HLA-DRB1*15:01 is approximately 8%, within the general range previously reported to be 8-20%.^{15,16}

This study found no statistically significant association between the Inv22 and HLA-DRB1*15:01 allele with inhibitor development in hemophilia A, either for the first inhibitor occurrence (Table 2) or at any point during treatment (Table 3), when compared to non-inhibitor hemophilia A patients, non-hemophilia A group, and normal controls. The results are consistent with previous studies, which reported Inv22 in 45.5% of hemophilia A patients with inhibitors (5/11), though this was not statistically significant when compared to

patients without inhibitors ($p=0.532$).¹⁴ These findings are consistent with previous studies indicating that the Inv22 gene is not significantly associated with inhibitor development.^{9,17} HLA-DRB1*15:01, DQB1*06:02, and DQA1*01:02 were frequently observed in hemophilia A patients with inhibitors. Notably, one study found a significant association for HLA-DQA1*01:02 (OR=2.7, 95% CI=1.2-4.9).¹⁸ Nathalang O. and coworker reported an increased likelihood of HLA-DRB1*15 in hemophilia patients with inhibitors compared to healthy controls (OR=2.74, 95% CI=1.16-6.47, $p=0.021$), but no statistical significance when comparing inhibitor-positive and inhibitor-negative hemophilia patients ($p=0.198$).⁶ Consequently, our study focused on HLA-DRB1*15:01 and Inv22 as primary risk factors in Thais; other alleles showed no significant association, likely due to ethnic genetic diversity. However, when examining the combined effect of the Inv22 and the HLA-DRB1*15:01 allele on inhibitor development, their co-occurrence was found to influence inhibitor formation at any point during treatment (Table 4). These findings regarding the influence of the Inv22 and HLA-DRB1*15:01 allele remain inconsistent, suggesting that while this allele may contribute to immunogenicity, it is not a sole determinant. Our results clarify that these inconsistencies likely stem from the complex synergy between genetic markers rather than isolated effects. By demonstrating that their co-presence significantly increases inhibitor risk throughout treatment (OR=7.90), this study highlights that a multi-genetic approach provides a more robust risk assessment than individual gene analysis alone. Taken together, these insights highlight the multifactorial nature of inhibitor formation, whereby both FVIII gene mutation severity and immune response genes intersect with additional clinical and environmental factors, such as treatment regimen and patient immune status. A better understanding of these genetic determinants will aid in identifying high-risk patients and guiding personalized therapeutic strategies, including prophylactic treatment and immune tolerance induction.⁹

Limitations

This study is limited by a relatively small sample size and the use of univariate analysis, which may not account for all clinical confounders. Additionally, we focused on a limited number of genetic markers (Inv22 and HLA-DRB1*15:01). Future studies with larger cohorts and broader genetic panels are necessary to validate these findings.

Conclusion

This study examined the association between intron 22 inversion (Inv22) and the HLA-DRB1*15:01 allele with inhibitor development in hemophilia A patients. The findings suggest that while Inv22 and HLA-DRB1*15:01 allele alone may not directly influence inhibitor formation, their combined presence may contribute to increased risk over time. Further studies

with larger cohorts are warranted to validate these findings and better understand the genetic mechanisms underlying inhibitor development in hemophilia A.

Ethical approval

This cross-sectional study was approved by the Human Research Ethics Committee of Thammasat University (Science) according to COA (266/2565) and Maharat Nakhon Ratchasima Hospital (No.129/2022).

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Chitpon Seeljunya: data collection, statistical analysis, writing: original draft, **Chollanot Kaset:** methodology, statistical analysis, data interpretation, **Sirinart Chomean:** conceptualization, methodology, investigation, data interpretation, writing: review and editing.

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References

- [1] Witmer C, Young G. Factor VIII inhibitors in hemophilia A: etiology and treatment. *Ther Adv Hematol.* 2013; 4(1): 59-72. doi: 10.1177/2040620712464379.
- [2] El-Sheikh A, El-Ghamrawy M, Ibrahim M. Evaluation of factor VIII inhibitors among children with hemophilia A: a single-center study. *Zagazig Univ Med J.* 2024; 30(8.1): 4104-9. doi: 10.21608/zumj.2023.210924.2821.
- [3] Chuansumrit A, Sosothikul D, Rungruntham T, Sasanakul P, Tapaneeyakorn S, On-Takrai J, et al. The success of the national universal health coverage for hemophilia care in Thailand. *Haemophilia.* 2021; 27(1): 69-80. doi: 10.1111/hae.14144.
- [4] Gouw SC, van den Berg HM, Oldenburg J, Astermark J, de Groot PG, Margaglione M, et al. F8 gene mutation type and inhibitor development in patients with severe hemophilia A: systematic review and meta-analysis. *Blood.* 2012; 119(12): 2922-34. doi: 10.1182/blood-2011-09-379453.

- [5] Oldenburg J, Picard JK, Schwaab R, Brackmann HH, Tuddenham EG, Simpson E. HLA genotype of patients with severe Haemophilia A due to Intron 22 inversion with and without inhibitors of Factor VIII. *Thromb Haemost.* 1997; 77(2): 238-42. doi: 10.1055/s-0038-1655945
- [6] Nathalang O, Sinsiriwong S, Pan-in T, O-charoen R, Chuansumrit A. Association between HLA-DRB1 alleles and FVIII inhibitors in Thai patients with severe hemophilia A. *Turk J Hematol.* 2012; 29(1): 34-9. doi: 10.5505/tjh.2012.87126.
- [7] Satapornpong P, Jantararoungtong T, Konyoung P, Khunkaew S, Sukasem C. Epidemiology of HLA-B*15:02 and other HLA alleles as pharmacogenetic markers in Thais. *Front Pharmacol.* 2020; 11: 78. doi: 10.3389/fphar.2020.00078.
- [8] Astermark J, Oldenburg J, Pavlova A, Berntorp E, Lefvert AK. Polymorphisms in the IL10 and TNF-alpha gene promoters and inhibitor development in patients with hemophilia A. *Blood.* 2006; 107(8): 3167-72. doi: 10.1182/blood-2005-09-3811.
- [9] Mancuso ME, Santagostino E. The role of emicizumab in hemophilia A patients with inhibitors. *J Thromb Haemost.* 2012; 10(5): 781-90. doi: 10.1111/j.1538-7836.2012.04683.x.
- [10] Verbruggen B. Diagnosis and quantification of factor VIII inhibitors. *Haemophilia.* 2010; 16(102): 20-4. doi: 10.1111/j.1365-2516.2010.02261.x.
- [11] Rossetti LC, Radic CP, Larripa IB, De Brasi CD. Genotyping of hemophilia A rearrangements using long-distance PCR. *J Thromb Haemost.* 2008; 6(5): 830-6. doi: 10.1111/j.1538-7836.2008.02927.x.
- [12] Delfan N, Galehdari H, Kazeminejad SR, Alikamali M, Ahmadi M. Analysis of HLA-DRB1*15:01 in multiple sclerosis patients: a case-control study. *Zahedan J Res Med Sci.* 2017; 19(3): e5647. doi: 10.5812/zjrms.5647.
- [13] Pavlova A, Delev D, Lacroix-Desmazes S, Schwaab R, Mende M, Fimmers R, et al. Impact of polymorphisms of the major histocompatibility complex class II on inhibitor development in severe hemophilia A. *J Thromb Haemost.* 2009; 7(12): 2006-15. doi: 10.1111/j.1538-7836.2009.03636.x.
- [14] Abu Arra CM, Arra IA, El-Gharbawy FA, Mansour S. Factor VIII intron 22 inversion in severe hemophilia A patients in Palestine. *Scientifica (Cairo).* 2020; 2020: 3428648. doi: 10.1155/2020/3428648.
- [15] Chuansumrit A, Sasanakul P, Seksarn P, Sosothikul D, Rungrungham T, Tapaneeyakorn S, et al. Genotype and phenotype of haemophilia A in Thai patients. *Haemophilia.* 2003; 9(2): 179-86. doi: 10.1046/j.1365-2516.2003.00727.x.
- [16] Romphruk AV, Romphruk A, Incheun R, Butthep P, Buaklee N, Jatuasitha S, et al. HLA class I and II alleles and haplotypes in ethnic Northeast Thais. *Tissue Antigens.* 2010; 75(6): 701-11. doi: 10.1111/j.1399-0039.2010.01449.x.
- [17] Abdulqader AMR, Mohammed AI, Rachid S, Ghoraishizadeh P, Mahmood SN. Identification of the intron 22 and intron 1 inversions of the factor VIII gene in Iraqi Kurdish patients with hemophilia A. *Clin Appl Thromb Hemost.* 2020; 26: 1076029619888293. doi: 10.1177/1076029619888293.
- [18] Hay CR, Ollier W, Pepper L, Cumming A, Keeney S, Goodeve AC, et al. HLA class II profile: a weak determinant of factor VIII inhibitor development in severe haemophilia A. *Thromb Haemost.* 1997; 77(2): 234-7. doi: 10.1055/s-0038-1655944.