Research Article

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The Association Between HLA Class II Alleles and the Occurrence of Factor VIII Inhibitor in Thai Patients with Hemophilia A

Tayland'lı Hemofili A Hastalarında, HLA Sınıf II Alleleri ve Faktör VIII İnhibitör Oluşumunun İlişkisi

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Abstract

Objective: This study aimed to investigate the association between HLA class II alleles and the occurrence of FVIII inhibitor in Thai hemophilia A patients.

Material and Methods: The distribution of HLA-DRB1 alleles and DQB1 alleles in 57 Thai hemophilia A patients and 36 blood donors as controls was determined using the PCR sequence-specific primer (PCR-SSP) method, and the association between the occurrence of factor VIII (FVIII) inhibitor and the presence of certain HLA class II alleles was investigated.

Results: The frequency of HLA-DRB1*15 was higher in the hemophilia A patients with and without FVIII inhibitor, whereas that of DRB1*14, DRB1*07, and DQB1*02 was lower in the hemophilia A patients with FVIII inhibitor, as compared to controls. Interestingly, only the frequency of DRB1*15 was significantly higher in the patients with inhibitor than in the controls (P = 0.021). Moreover, the frequency of DRB1*15 in the patients with inhibitor was higher than in those without inhibitor (P = 0.198).

Conclusion: The study's findings show that the DRB1*15 allele might have contributed to the occurrence of inhibitor in the Thai hemophilia A patients; however, additional research using larger samples and high-resolution DRB1 typing is warranted.

Key Words: HLA class II alleles, FVIII inhibitor, Hemophilia A, Thais

Özet

Amaç: Bu çalışmada HLA sınıf II allelleri hemofili A ile Tayland'lı hastalarda FVIII inhibitörü oluşumu ile ilişkiyi araştırmaktır.

Gereç ve Yöntemler: Hemofili 57 Tayland'lı hastalarda HLA-DRB1 allelleri ve DQB1 allel dağılımı bir PCR-sıra özel astar (PCR-SSP) yöntemi ve faktör VIII (FVIII) inhibitör oluşumu arasındaki ilişki kullanılarak belirlendi ve bazı HLA klas II allelleri varlığı araştırılmıştır.

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Bulgular: HLA-DRB1 sıklığı * 15 ve FVIII inhibitörü olmayan iki hemofili hastalarında artmış iken DRB1 olanlar * 14, DRB1 * 07 ve DQB1 * 02 FVIII inhibitörleri ile hemofili hastalarında azalmıştı. İlginçtir, sadece DRB1 * 15 önemli kontrol (P = 0.021) ile karşılaştırıldığında inhibitörleri olan hastalarda artmıştır. Ayrıca, inhibitörü olan hastalarda DRB1 * 15 frekans olmayan inhibitörü (P = 0.198) olan hastalarda daha yüksek olma eğiliminde.

Sonuç: Bulgularımız DRB1 * 15 allel Tay hemofili A hastalarında inhibitör oluşumuna katkıda bulunabileceğini göstermiştir. Ancak, daha büyük bir örneklem büyüklüğü ve yüksek çözünürlüklü DRB1 yazarak garantilidir.

Anahtar Sözcükler: HLA klas II allelleri, FVIII inhibitörü, Hemofili A, Thais

Introduction

One of the most serious complications in patients with hemophilia A is the occurrence of an inhibitor frequently, IgG4 antibodies directed against epitopes in factor VIII (FVIII). This may be induced by replacement of the missing factor via cryoprecipitate or FVIII concentrate administration. The antibody attached to FVIII will neutralize or inhibit its ability to stop bleeding. FVIII inhibitor is usually detected in one of two ways. First, the inhibitor may be discovered in asymptomatic patients via routine screening performed during a comprehensive clinical examination. Second, an inhibitor may be detected when bleeding is suddenly and unexpectedly unresponsive to treatment with FVIII. Generally, the incidence of FVIII inhibitor in hemophilia A patients with severe disease (FVIII:C <1%) and moderate disease (FVIII:C >1% to 5%) is estimated to be 20% and 33%, respectively [1-6]; however, differences in the incidence between ethnic groups might be due to genetic differences. Moreover, the lack of recognition is one of the causes of low incidence of inhibitor in economically less-developed countries.

It has been reported that molecular defects in the factor VIII gene and the major histocompatibility complex molecules, especially HLA class II alleles, are associated with antibody formation. An increased occurrence of FVIII inhibitor was reported in cases of severe congenital hemophilia A with HLA-DRB1*15:01, DQA1*01:02, and DQB1*06:02 alleles [8,9]. Conversely, another study reported that HLA class I alleles were not associated with the occurrence of FVIII inhibitor in patients with acquired hemophilia A, whereas DRB1*16 and DQB1*0502 were associated with a high risk of such an occurrence in hemophilia A patients with FVIII inhibitor [10]; however, the association between these alleles and FVIII inhibitor in Thai patients with hemophilia A remains unknown. As such, the present study aimed to investigate the association between HLA class II alleles and the occurrence of FVIII inhibitor in a group of Thai patients with hemophilia A.

Material and Methods

The study included 57 hemophilia A patients from Mahidol University, Faculty of Medicine Ramathibodi Hospital, Division of Hematology, Department of Pediatrics, Bangkok, Thailand, and a control group consisting of 36 unrelated male blood donors from the National Blood Center of the Thai Red Cross Society. The study protocol was approved by the Mahidol University, Faculty of Medicine Ethics Committee and the Committee on Human Rights Related to Research Involving Humans. Informed consent was obtained from each participant and/or his parents.

The patients were regularly monitored for factor VIII inhibitor every 6-12 months or when clinically indicated in cases of unresponsiveness to replacement therapy. The inhibitor titer against human factor VIII clotting activity was determined via the Bethesda method [11]. A Bethesda unit (BU) level >0.6 was considered indicative of the presence of inhibitor. Moreover, genetic defect associated with hemophilia A was carried out. Inversion of intron 22 was initially determined via inverse polymerase chain reaction (PCR) [12,13]. In patients without inversion of intron 22 conformation-sensitive gel electrophoresis was used to further investigate the genetic defect [14], followed by sequencing.

Genomic DNA was extracted from peripheral blood cells using the salting out technique [15]. The second exon of the DRB1 and DQB1 genes was amplified using the PCR-SSP method. Each DNA sample (100 ng μL^{-1}) was tested using a Micro SSP Generic HLA Class II Typing Kit (One Lambda Inc., Canoga Park CA, USA). Briefly, for HLA class II low-resolution typing each DNA sample (100 ng) was amplified with 31 different primer sets optimized and dispensed into each well of a 96-well thin-walled PCR plate. The SSP-DNA reaction set was placed in a G-STORM GS1 thermal cycler (Gene Technologies Ltd., Essex, UK). The cycle parameters of the PCR program were set according to the manufacturer's instructions. The reaction pattern was photographed and HLA alleles were assessed via

analysis of the gel banding pattern using a reaction pattern typing grid.

The association of the HLA class II alleles and the development of an inhibitor in Thai patients with hemophilia A was calculated using the odds ratio (OR) and 95% confidence interval (CI). The frequency of alleles in the patients and controls was compared using chi-square contingency table analysis with Yates' correction, as well as standard P values and Fisher's exact test. A P value <0.05 was accepted as statistically significant.

Results

The study included 57 male Thai hemophilia A patients with a mean age of 14.4 ± 8.9 years. The patients were divided into 2 groups: 26 patients without inhibitors and 31 patients with a high inhibitor titer ≥5 BU (n = 22), low inhibitor titer <5 BU (n = 3), and transient low inhibitor titer for <6 months (n = 6). The mean high inhibitor titer was 540.9 BU (range: 5.3-3920 BU) and the mean low titer was 3.3 BU (range: 2.9-4.2 BU), whereas the mean transient low titer was 2.0 BU (range: 1.0-3.3 BU).

A molecular defect related to the factor VIII gene was observed in 35 of the 57 patients (61.4%) of which 15 were in the non-inhibitor group and 20 were in the inhibitor group. In all, 6 patients in the non-inhibitor group and 12 patients in the inhibitor group had inversion of intron 22: however, the difference in the number of patients with inversion between the patients with and without inhibitor was not statistically significant (P = 0.32). The specific mutations were investigated in the 17 patients without inversion of intron 22; 10 patients had point mutations and mutations could not be identified in the other 7 patients. Interestingly, point mutations inducing stop codon (n = 2), amino acid alteration (n = 2), and frameshift mutation (n = 1) were observed in patients without inhibitor, and point mutations inducing stop codon (n = 5) were noted in patients with inhibitor. The occurrence of stop codon in patients with inhibitor was higher than those without inhibitor (P = 0.05).

The distribution of HLA-DRB1 and DQB1 alleles, according to PCR-SSP low-resolution typing, in the patients with and without inhibitor, and in the controls is shown in Tables 1 and 2. Overall, 13 DRB1 alleles were noted in the hemophilia A patients, of which DRB1*15 and DRB1*12 were the most frequent; additionally, 7 DQB1 alleles were identified. The most common DQB1 alleles in the patients

Table 1: Distribution of HLA-DRB1 Alleles in the Thai Hemophilia A Patients and Controls

DRB1 allele	Hemophilia A without inhibitor (n = 26)		Hemophilia A with inhibitor (n = 31)		Controls (n = 36)	
	Observed	%	Observed	%	Observed	%
DRB1*01	1	1.9	0	0.0	1	1.4
DRB1*03	2	3.8	1	1.6	5	6.9
DRB1*04	5	9.6	6	9.7	10	13.9
DRB1*07	5	9.6	3	4.8	10	13.9
DRB1*08	4	7.7	0	0.0	6	8.3
DRB1*09	5	9.6	6	9.7	3	4.2
DRB1*10	0	0.0	1	1.6	2	2.8
DRB1*11	4	7.7	2	3.2	4	5.6
DRB1*12	7	13.5	13	21.0	8	11.1
DRB1*13	1	1.9	5	8.1	4	5.6
DRB1*14	8	15.4	4	6.5	6	8.3
DRB1*15	10	19.2	19	30.6**	10	13.9
DRB1*16	0	0.0	2	3.2	3	4.2

***P* = 0.021; *OR* = 2.74; 95% *CI* = 1.16- 6.47.*

Table 2: Distribution of HLA-DQB1 Alleles in the Thai Hemophilia A Patients and Controls

DQB1 allele	Hemophilia A without inhibitor (n = 26)		Hemophilia A with inhibitor (n = 31)		Controls (n = 36)	
	Observed	%	Observed	%	Observed	%
DQB1*05	18	34.6	22	35.5	19	26.4
DQB1*06	7	13.5	10	16.1	11	15.3
DQB1*02	7	13.5	3	4.8	13	18.1
DQB1*03:01/:04	9	17.3	14	22.6	13	18.1
DQB1*03:02/:05/:07	3	5.8	2	3.2	5	6.9
DQB1*03:03:02/:06	5	9.6	7	11.3	6	8.3
DQB1*04	3	5.8	4	6.5	5	6.9

and controls were DQB1*05 and DQB1*06, respectively. DQB1*03 was sub-typed as DQB1*03:01/03:04, DQB1*03:02/03:05/03:07, and DQB1*03:03:02/03:06 via PCR-SSP low-resolution typing.

The frequency of DRB1*15 was higher in the patients (both with and without inhibitor) than in the controls, however, statistical significance was found between patients with inhibitor and the controls (30.6% vs. 13.9%; P = 0.021; OR = 2.74; 95% CI = 1.16-6.47). The frequency of DRB1*15 in the patients with FVIII inhibitor (30.6%) was higher than that in the patients without inhibitor (19.2%), but the difference was not statistically significant (P = 0.198). On the other hand, the frequency of DRB1*14, DRB1*07, and DQB1*02 was lower in the patients with inhibitor than in those without inhibitor (P > 0.05).

Discussion

Both genetic and non-genetic risk factors have been implicated in the development of factor VIII inhibitor [16,17]. The molecular defects in the FVIII gene that cause a defect in translation and protein production is a primary cause of inhibitor formation. Polymorphisms associated with HLA class II molecules, interleukin-10 (IL-10), and tumor necrosis factor-α (TNF-α) also influence to the FVIII inhibitor development [18]. Immunological mechanisms in the cellular processing of peptide antigens are involved in the development of inhibitor in patients with hemophilia A. Moreover, the major histocompatibility complex phenotype is also involved in inhibitor formation [19-21].

Although a molecular defect associated with factor VIII was observed in 35 of the 57 patients in the present study, inversion of intron 22 was observed in more of the patients with inhibitor than in those without inhibitor, as previously reported [22,23]; however, the frequency of point mutations causing stop codon in patients with inhibitor was significantly higher than in those without inhibitor, which was also previously reported [24]. Moreover, the association between HLA class II alleles and the FVIII inhibitors in Thai hemophilia patients was further investigated in this study, as recent studies have indicated that inhibitor formation depends upon an adequate T-cell response by major histocompatibility complex class II molecules to FVIII resulting from the presentation of FVIII protein antigen to T-cell receptors [18-21]. It was reported that in mild hemophilia A patients with inhibitor the frequency of DRB1*01 and DQB1*05 was slightly higher than the controls (but not significantly) [25]. A comparison of the frequency data for DRB1*15/16 in hemophilia A patients with FVIII inhibitor reported in other studies showed that DRB1*15 and DRB1*16 were high-risk alleles for inhibitor formation in patients with congenital hemophilia A and acquired hemophilia A, respectively [8-10].

The DRB1*15 allele is known to exhibit the specific surface loop peptide comprising amino acids 1706-1721 of the FVIII light chain, and is considered to be involved in FVIII inhibitor formation in patients with congenital hemophilia A that lack endogenous FVIII protein synthesis [8,26]. Because the ability to recognize and process FVIII peptides is determined by the number of HLA class II molecules in each individual. It was reported that

there are as many as 13 potential recognition sequences for HLA-DRB1*1501 in FVIII, whereas there are only 2 recognition sequences for HLA-DRB1*1101 [27]. Even though HLA-DRB1*15 (17.5%), DRB1*12 (16.9%), and DRB1*09 (11.5%) were the most common in Thai blood donors [28], the frequency of the DRB1*15 allele among hemophilia A patients with inhibitors in the present study was significantly higher than in the controls.

Limitations of the present study included the small number of patients enrolled, incomplete detection of molecular defects of the factor VIII gene, and the lack of exploration of the polymorphisms associated with IL-10 and TNF-α. In conclusion, the DRB1*15 allele may have contributed to inhibitor formation in Thai patients with hemophilia A. Additional comprehensive research with larger patient populations is warranted.

Conflict of Interest Statement

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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