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Original Article

Association of HLA class II and history of atopy and frequent relapse of childhood steroid-sensitive nephrotic syndrome

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Abstract

Background The association between HLA class II and frequent relapse of nephrotic syndrome (FRNS) has been reported.

Objective To identify the association between HLA class II, history of atopy, and upper respiratory tract infection (URTI) with FRNS.

Methods This was a case control study conducted at the Department of Child Health, Hasan Sadikin Hospital Bandung and Cipto Mangunkusumo Hospital Jakarta from November 2002 to October 2003 on children aged 1-14 years with FRNS. The subjects consisted of 40 FRNS and 84 healthy children. HLA class II was typed by polymerase chain reaction-sequence specific oligonucleotide (PCR-SSO) in Leiden, the Netherlands. The association between HLA class II and FRNS was expressed by odds ratio (OR). The association between such factors and FRNS was analyzed by logistic regression.

Results Atopy was higher in patients than that in controls (P=0.013). URTI did not differ in both groups (P=0.173). HLA-DRB1*03 and DRB1*04 (OR=4.43, P=0.03), DQB1*02 (OR=3.43, P=0.00), and DQB1*04 (OR=12.06, P=0.01) were significantly higher among patients than those in controls whereas HLA-DRB1*12 (OR=0.34, P=0.02) and DQB1*0301p (OR=0.35, P=0.02) were significantly lower among patients than those in controls. Using logistic regression analysis, only HLA-DRB1*12, DQB1*02 and atopy took part in FRNS.

Conclusion HLA-DRB1*12, DQB1*02, and atopy all together have association with FRNS. **[Paediatr Indones 2007;47:60-64]**.

Keywords: *Frequent relapsing nephrotic syndrome, risk factor, Malay ethnic, PCR-SSO*

Most children with nephrotic syndrome respond to steroid treatment. However, approximately 60 to 80% of childhood nephrotic syndrome experience relapses; half of them are frequent relapsers or steroid dependent.[1] It is well known that human leukocyte antigen (HLA) is associated with the occurrence of frequent relapse of childhood steroid sensitive nephrotic syndrome (SSNS).[2-5] It is important to know the association between HLA and the occurrence of frequent relapse of nephrotic syndrome (FRNS) since high and repeated doses of steroid usually accompanied by severe steroid toxicity.[6-7]

Association of HLA class II with FRNS was reported by previous studies in different populations.[2,4] Because HLA has high polymorphisms and varies among races, it is believed that HLA class II will also differ in Indonesian population. Besides HLA, other factors are associated with FRNS, atopy,[8,9] age of onset,[10,11] upper respiratory tract infection (URTI),[6,12] total plasma protein level,[13] and time to achieve remission in the initial attack of

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the disease.[6,14] However, the controls of those studies were healthy children without history of nephrotic syndrome, so the included risk factors were history of atopy and URTI. This study aimed to determine whether types of HLA class II, history of atopy, and URTI were risk factors for FRNS.

Methods

This was a case control study conducted at Department of Child Health, Hasan Sadikin Hospital Bandung and Cipto Mangunkusumo Hospital Jakarta from November 2002 to October 2003. This study was approved by the Ethics Committee of Hasan Sadikin Hospital.

Subjects aged 1-14 years with FRNS were selected consecutively. Patients were diagnosed as FRNS according to criteria of the International Study of Kidney Disease in Children (ISKDC). Nephrotic syndrome is the presence of severe proteinuria, edema, and albumin serum level of <2.5 g/dL. Frequent relapse of nephrotic syndrome was defined as a condition with two or more relapses within six months from initial response. The control group consisted of healthy children aged 1 to 14 years who came to paediatric division of Hasan Sadikin Hospital Bandung and Cipto Mangunkusumo Hospital Jakarta. History of atopy was obtained by questioning whether they had any history of atopy diseases in parents or subjects.

Venous blood samplings were drawn from every subject. DNA extraction for HLA class II typing were performed by Medical Research Unit of the Padjadjaran University Bandung and HLA typing using polymerase chain reaction-sequence specific oligonucleotide (PCR-SSO) performed in Department of Immunobiology and Blood Transfusion, Leiden University Medical Center, the Netherlands.

Chi square with Yates correction was used to determine the association between HLA class II in FRNS and control groups. Odds ratio was calculated to show the association between FRNS and HLA class II. Logistic regression analysis was used to assess significant association between FRNS and HLA class II with other risk factors (history of atopy and URTI). The possible interaction among various risk factors with FRNS were assessed. P value of <0.05 was considered as significant.

Results

Fourty children with FRNS and 84 healthy children as controls were enrolled in this study. One child in each group was excluded because their HLA class II could not be typed from their DNA extraction. All subjects were Malay ethnics.

HLA-DR and DQ genotype were typed in FRNS and control groups. HLA class II frequencies of patients with frequent relapses/steroid dependent nephrotic syndrome and healthy controls are shown in **Table 1**.

The proportions of HLA class II type DRB1*03, DRB1*04, DRB1*12, DQB1*02, DQB1*0301p, and DQB1*04 were significantly different between FRNS and controls (P<0.05). HLA-DRB1*03 (OR=4.43; P=0.035), DRB1*04 (OR=4.43; P=0.035), DQB1*02 (OR=3.43; P=0.003), and DQB1*04 (OR=12.06; P=0.013) alleles frequency were significantly higher among patients compared to controls, whereas DRB1*12 (OR=0.34; P=0.015) and DQB1*0301p (OR=0.35; P=0.018) were significantly higher among controls compared to patients.

This study showed that history of atopy was significantly higher among patients compared to that of controls (P=0.013) (**Table 2**).

Table 1. Frequency of HLA class II in FRNS patients and controls

HLA	FRNS patients		Controls		OR	P*
	Positive	Negative	Positive	Negative		
DRB1*01	1	36	0	80	0.00	0.3162
DRB1*15	22	15	47	32	1.00	1.0000
DRB1*16	0	37	1	78	0.00	1.0000
DRB1*03	7	30	4	76	4.43	0.0350
DRB1*04	7	30	4	76	4.43	0.0350
DRB1*11	4	33	2	78	4.73	0.0786
DRB1*12	9	28	39	41	0.34	0.0154
DRB1*13	0	37	6	74	0.00	0.1747
DRB1*14	4	33	6	74	1.50	0.7233
DRB1*07	12	25	15	65	2.08	0.1556
DRB1*08	3	34	2	78	3.44	0.3242
DRB1*09	1	36	2	78	1.08	1.0000
DRB1*10	1	36	2	78	1.08	1.0000
DQB1*05	23	16	42	41	1.40	0.4395
DQB1*06	9	30	29	54	0.56	0.2140
DQB1*02	19	20	18	65	3.43	0.0033
DQB1*0301p	10	29	41	42	0.35	0.0178
DQB1*0302p	6	33	4	79	3.60	0.0731
DQB1*0303p	1	38	5	78	0.41	0.6631
DQB1*04	5	34	1	82	12.06	0.0126

Table 2. Distribution of history of atopy in FRNS patients and controls

	Atopy	No Atopy	95%CI	P*
FRNS	24(61.5%)	15(38.5%)	1.1-3.2	0.013
Controls	30(36.1%)	53(63.9%)	0.6-1.0	

Note: 95%CI = 95% confidence interval, P* student t test

The results of this study indicated that HLA-DRB1*03, DRB1*04, DRB1*12, DQB1*02, DQB1*04, DQB1*0301p, and history of atopy had significant roles in the occurrence of FRNS. After multivariate analysis, only HLA-DRB1*12, DQB1 *02, DQB1*04 and history of atopy had roles in FRNS. Types of HLA either alone or in combination, that might be assembled from HLA-DRB1*12, DQB1*02, and DQB1*04 is shown in **Table 3**.

There was no sample with HLA-DQB1*04 only, neither combination of HLA-DQB1*02 and DQB1*04, nor combination of HLA-DRB1*12, DQB1*02, and DQB1*04. Based on P value in **Table 3**, only HLA-DRB1*12 and DQB1*02 that could be included in multivariate analysis (**Table 4**).

Adjusted R^2 of 0.21 indicated that the role of HLA-DRB1*12, DQB1802, and history of atopy simultaneously to FRNS was 21%. However, there was no interaction among those factors in their role to FRNS.

Table 3. HLA type that might be formed from HLA-DRB1*12, -DQB1*02 and –DQB1*04

Variable	FRNS Patients (n=39)	Controls (n=83)	P
HLA-DRB1*12 only	7(17.9%)	39(47.0%)	0.002*
HLA-DQB1*02 only	18(46.1%)	17(20.5%)	0.004*
HLA-DRB1*12 and -DQB1*02	2(5.0%)	5(6.0%)	1.000**
HLA-DRB1*12 and –DQB1*04	2(5.0%)	0(0%)	0.102**

*Chi square test **Fisher exact test

Table 4. Multivariate analysis of factors associated with FRNS

Variable	b	P	Adjusted R^2
Constant	-1.712	0.029	
HLA-DRB1*12 only	-1.447	0.002	
HLA-DQB1*02 only	1.162	0.004	0.21
History of atopy	1.176	0.013	

Discussion

In the present study we reported that HLA-DQB1*02 and history of atopy were the risk factors of FRNS. In univariate analysis, we also found the other risk factors for FRNS. However, after doing multivariate analysis, those factors were excluded. Our study also revealed HLA-DRB1*12 and DQB1*0301p as the protective factors for FRNS. However, by multivariate analysis, only HLA-DRB1*12 had role in the occurrence of FRNS.

The association between FRNS and HLA class II found in our study, not only supported the predisposition of genetic factor in FRNS, but also showed that there is the disturbance of immune response in the disease.

Our study demonstrated that the pattern of HLA class II in relationship with FRNS were different with other populations. In the present study we found that HLA-DRB1*03, DRB1*04, DQB1*02, and DQB1*04 were higher in FRNS patients than those in controls. Konrad *et al*[15] reported that the child with combination of HLA-B8, DR3, and DR7 had high risk to become FRNS or steroid dependent nephrotic syndrome. Different result of another population was found by Zhou *et al*[3] on Chinese children. Zhou *et al*[3] showed the higher frequency of HLA-DR9 in FRNS patients compared to that of healthy children (relative risk or RR=6.64, P=0.016).

The association between HLA-DQ and FRNS had been revealed by previous studies. Our study showed similar result with Bouissou *et al*[4] who found higher frequency of HLA-DQ2 in children with FRNS compared to controls.

The present study showed that HLA-DRB1*12 and DQB1*0301p were the protective factors for FRNS. Previous study by Haeffner *et al*[5] revealed HLA-DR2 as the protective factor for FRNS (RR=0.2, P<0.0003). Whereas Bouissou *et al*[4] demonstrated HLA-DQ1 as the protective factor for FRNS (RR=0.1, P<0.00001).

It is believed that genetic factors play important role in immunological disease like nephrotic syndrome. According to Campbell and Trowsdale, cited by Haeffner[5], the most important type of HLA in association with immunological disease were HLA-DR and DQ. This is because of the unique amino acids

contained in HLA-DR and DQ that easily allowed attachment of peptides into cleave of HL.

The present study revealed that there was a high incidence of history of atopy in FRNS patients. This result was similar with the previous studies. Trompeter *et al*[8] showed that relapses in nephrotic syndrome patients was associated with atopy disease and also the high level of serum IgE, mainly in frequent relapsers. Study by Meadow *et al*[9] also reported that atopy disease has hereditary tendency. Atopy disease has a close relationship with certain type of HLA, particularly HLA-DR7. The correlation between atopy and relapses of nephrotic syndrome, genetically remain unclear. However, it might be mediated by HLA-DR7.

Our study did not show the correlation between acute URTI and FRNS. Yap *et al*[6] reported that the mechanisms of relapsed nephrotic syndrome following acute viral URTI not fully understood. However, viral infection may stimulate the release of cytokines that could upset the regulation of immune response in nephrotic syndrome patients, thereby causing relapses.

This study revealed the correlation between HLA-DRB1*12, DQB1*02 together with history of atopy and FRNS. The statistical analysis showed the adjusted R^2 was 0.21. This indicated that the role of HLA-DRB1*12, DQB1*2, history of atopy, and FRNS was 21%. Our study also demonstrated that the risk factors or protective factors of FRNS did not exert discretely, but rather collectively to play a role in FRNS. However, our study did not show any interaction among the risk factors in relation with the occurrence of FRNS.

In conclusion, the types of HLA as the risk factors of FRNS in our study were HLA-DRB1*03, DRB1*04, DQB1*02, and DQB1*04. Whereas HLA-DRB1*12 and DQB1*0301p were the protective factors for FRNS. However, only HLA-DRB1*12 and DQB1*02 had role in the occurrence of FRNS when the history of atopy were included in multivariate analysis.

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References

1. International Study of Kidney Disease in Children. Early identification of frequent relapsers among children with minimal change nephrotic syndrome. J Pediatr 1978; 101:514–8.
2. Konrad M, Mitilineos J, Bouissou F, Scherer S, Gulli MP, Meissner I, *et al*. HLA class II associations with idiopathic nephrotic syndrome in children. Tissue Antigens 1994; 43:275–80.
3. Zhou GP, Guo YQ, Ji YH, Zhang GL. Major histocompatibility complex class II antigen in steroid-sensitive nephrotic syndrome in Chinese children. Pediatr Nephrol 1994;8: 140–1.
4. Bouissou F, Meissner I, Konrad M, Sommer E, Mytilineos J, Ohayon E, *et al*. Clinical implications from studies of HLA antigens in idiopathic nephrotic syndrome in children. Clin Nephrol 1995;44:279–83.
5. Haeffner A, Abbal M, Mytilineos J, Konrad M, Krammer I, Bouissou F, *et al*. Oligotyping for HLA-DQA, DQB, and DPB in idiopathic nephrotic syndrome. Pediatr Nephrol 1997;11:291–5.
6. Yap HK, Han EJS, Heng CK, Gong WK. Risk factors for steroid dependency in children with idiopathic nephrotic syndrome. Pediatr Nephrol 2001;16:1049-52.
7. Donia AF, Amer GM, Ahmed HA, Gazareen SH, Moustafa FE, Shoeib AA, *et al*. Levamisole:adjunctive therapy in steroid dependent minimal change nephrotic syndrome. Pediatr Nephrol 2002;17:355–8.
8. Trompeter RS, Barratt TM, Kay R, Turner MW, Soothill JF. HLA, atopy, and cyclophosphamide in steroid-responsive childhood nephrotic syndrome. Kidney Int 1980;17:113–7.
9. Meadow SR, Sarsfield JK. Steroid-responsive nephrotic syndrome and allergy: clinical studies. Arch dis Child 1981;56:509–16.
10. Kabuki N, Okugawa T. Influence of age at onset on the outcome of steroid-sensitive nephrotic syndrome. Pediatr Nephrol 1998;12:467–70.
11. Takeda A, Takimoto H, Mizusawa Y, Simoda M. Prediction of subsequent relapse in children with steroid-sensitive nephrotic syndrome. Pediatr Nephrol 2001;16:888–93.
12. Haycock GB. Steroid-responsive nephrotic syndrome. In: Postlethwaite RJ, editor. Clinical pediatric nephrology. Oxford: Butterworth-Heineman, 2003; p. 253–9.
13. Takeda A, Matsutani H, Niimura F, Ohgushi H. Risk factors for relapse in childhood nephrotic syndrome. Pediatr Nephrol 1996;10:740–1.

14. Constantinescu AR, Shah HB, Foote EF, Pharm D, Weiss LS. Predicting first year relapses in children with nephrotic syndrome. Pediatrics 2000;105:492–5.

15. Konrad M, Mytilineos J, Ruder H, Opelsz G, Scharer K. HLA-DR7 predicts the response to alkylating agents in steroid-sensitive nephrotic syndrome. Pediatr Nephrol 1997;11:16–9.