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Original Article

HLA-DRB1 alleles in Children with post-streptococcal acute glomerulonephritis

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Abstract: To investigate the association between HLA-DRB1 alleles and post-streptococcal acute glomerulonephritis (PSAGN), 32 children with PSAGN and 380 healthy subjects from the same locality were typed for DRB1 alleles using polymerase chain-reverse hybridization technique.
Patients with PSAGN have significantly increased frequency of both DRB1* 03011 (46.9 vs. 19.2% in controls, P = 0.00025) and DRB1* 1105 (31.1 vs. 15.6% in controls, P = 0.0097). However, after correction of P values, only the difference for DRB1* 03011 remains significant (Pc = 0.025). Their relative risks are significantly high [3.71, confidence interval (CI) = 1.8-7.8, and 3.57, CI = 1.4-8.9 respectively]. No significant differences in the frequency of both alleles are observed among patients with different grades of hypertension, proteinuria or hematuria.
Conclusions: DRB1* 03011, and presumably 1105, alleles confer susceptibility to PSAGN. However the severity of the disease is not determined by these two alleles.

Key words: Case-control study; DRB1; HLA; Poststreptococcal acute glomerulonephritis.

Introduction

Post - streptococcal acute glomerulonephritis (PSAGN) is a non-suppurative sequaele of infection by β -hemolytic streptococci. It is accepted that the immune response of the host is the central pathogentic event in the development of PSAGN [1] and the antibody response to streptococcal antigens is under genetic control [2]. Many researches have studied the association of PSAGN and serologicaly typed HLA antigens [3-5]. However, no consistent association has been found.
HLA class II antigens (DR, DQ, and DP) are hetrodimeric glycoproteins consisting of α and β chains. The genes coding for these antigens are located on the short arm of chromosome 6 [6]. HLA-DR has one gene coding for the DR α chain, DRA, and one gene coding for the β chain, DRB. Depending on the DRB type, DRB1 is always present, a DRB3, DRB4 or DRB5 gene may be present as well, eventually accompanied by pseudogenes [7]. DRA is not polymorphic, while DRB1 is highly polymorphic, and some polymorphism is described in DRB3, DRB4, and DRB5.

All these genes consist of 7 exons. Most of the allelic polymorphism is located in exon 2, encoding

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the amino-terminal extracellular domain functions as the antigen binding site for processed proteins.
This high polymorphism of DRB1 genes makes them powerful markers for population genetic studies [8].
Since DRB1 typing by molecular genetic techniques declares more specific association with diseases and no reports describe DRB1 alleles association in children with PSAGN in our population, we decided to study this association using DNA-polymerase chain-reverse hybridization technique.

Subjects

Thirty-two patients with PSAGN were included: 12 males, 20 females, aged 4-10 years. The diagnosis of PSAGN depends on the presence of hematuria, transient hypocomplementemia, and evidence of preceding streptococcal infection. The severity of hypertension was assessed according the task force on blood pressure in children [9]. Twenty-one patients had severe hypertension, 7 patients had non-severe, while 4 patients had normal blood pressure. Hematuria was microscopic in 18 patients and macroscopic in 14 patients. Twenty patients had nephrotic proteinuria (more than $1g/m^2/d$) while 12 patients had non-nephrotic range proteinuria. Patients were recruited from Division of Pediatric Nephrology, Mansoura Faculty of Medicine, Mansoura, Egypt from December 1996 to July 1997. The study was approved by the ethics committee of Mansoura Faculty of Medicine. Informed consent was obtained from the parents.

HLA class II alleles typing: Patients and controls were typed for DRB1 alleles by using INNO-LiPA, reverse hybridization line probe assay (Innogenetics. N.V. Belgium). The INNO-LiPA HLA typing tests are based on the reverse hybridization principle [10, 11]. DNA is at first extracted from the test sample, then amplified using polymerase chain reaction (PCR) [12]. The amplified biotinylated DNA material is chemically denatured, and the single strands are hybridized with specific oligonucleotide probes immobilized as parallel lines on membrane-based strips. After hybridization, streptavidin labeled with alkaline phosphatase is added and bound to any biotinylated hybrid previously formed. Incubation with chromogen results in purple brown precipitate. The reaction is stopped by a wash step and the reactivity pattern of the probes is recorded. The site and the molecular weight of the hybridized probe define a certain allele.

Statistical analyses: The results of HLA typing were compared to 380 unrelated healthy controls These subjects included persons coming to the hospital for minor surgical problems and parents of the children attending the pediatric outpatient clinic. SPSS computer package (release 5.0.2) was used for statistical analysis. The significance of association was tested using the χ^2 with continuity correction or two-tail Fisher's exact test. P values were corrected (Pc) by multiplying by the number of alleles tested (99 alleles).

The relative risk (RR) was calculated by Odd's ratio. The significance of RR was tested by total χ^2 [13]. Multiple $\chi^2 = WY^2$, where Y = Log e RR, and $W = 1/(1/h + 1/k + 1/H + 1/K)$. Where h is the number of patients having the antigen, k is number of patients lacking this antigen. H is the number of controls having the antigens and K is the number of controls lacking it. The etiologic fraction (EF) was calculated by the formula of Bengsson and Tomson. EF = (FAD - FAP) / (1 - FAP), where FAD denotes the frequency of the antigen in individuals with the disease, FAP is the frequency of the antigen in general population [14].

Results

Table 1 shows that patients with PSAGN have increased frequency of DRB1 *03011 allele (46.9 vs 19.2% in controls, P=0.0002, Pc=0.025). RR is high (3.71; CI=1.8-7.8) and significant (table 2).
EF is high (0.34). Although the frequency of DRB1* 1105 is higher in patients with PSAGN (31.1 vs 15.6% in controls, P= 0.0097), this frequency becomes non-significant when P value was corrected to the number of alleles tested. However its RR is still high (3.57; CI= 1.4-8.9) and significant (table 2).

Table 3 shows that patients with different grades of hypertension have no difference in the frequency of both DRB1* 03011 and 1105. Patients with microscopic or macroscopic hematuria have similar frequency of both DRB1* 03011 and 1105 (Fig. 1).

Similarly, no difference in the frequency of these two alleles between patients with different grades of proteinuria (Fig. 1).

Table 1. DRB1* allele frequencies in patients with poststreptococcal acute glomerulonephritis and controls

Allele	*Patients (n= 32)*		*Controls (n= 380)*		*P*	*Pc*	*RR*	*95% CI*	*EF*
	N	%	n	%					
DRB1* 1601	1	3.1	9	2.4					
DRB1* 16020	1	3.1	4	1.1					
DRB1* 03011	15	46.9	73	19.2	0.00025	0.025	3.71	1.8-7.8	0.34
DRB1* 0302	3	9.4	12	3.2					
DRB1* 0401	1	3.1	4	1.1					
DRB1* 0402	6	18.8	36	9.5					
DRB1* 0404	1	3.1	21	5.5					
DRB1* 0405	1	3.1	17	4.5					
DRB1* 0412	2	6.3	2	0.5					
DRB1* 0417	2	6.3	1	0.3					
DRB1* 11011	1	3.1	65	17.1					
DRB1* 1417	1	3.1	2	0.5					
DRB1* 0701	6	18.8	67	17.6					
DRB1* 09011	3	9.4	9	2.9					
DRB1* 1105	8	31.1	21	15.6	0.0097	0.96	3.57	1.4-8.9	0.18
DRB1* 1106	1	3.1	2	0.5					
DRB1* 1107	1	3.1	8	2					
DRB1* 1201	1	3.1	5	1.3					
DRB1* 1301	3	9.4	50	13.2					
DRB1* 1303	4	12.5	24	6.3					
DRB1* 1309	2	6.3	3	0.8					

Pc P corrected RR Relative risk CI Confendence interval EF etiologic fraction

Table 2. The significance of association of PSAGN and DRB1 alleles

	1/h	*1/k*	*1/H*	*1/K*	*y*	wy^2	*P*
DRB1* 03011	1/15	1/17	1/73	1/307	1.31	12.1	< 0.0001
DRB1* 1105	1/8	1/24	1/21	1/359	1.27	7.4	< 0.001

Y = Log e RR, W = 1/(1/h+1/k+1/H+1/K), h = No of patients having the antigen, k is No of patients lacking this antigen. H is No the number of controls having the antigens and K is No of controls lacking it

Table 3. Frequency of DRB1* 03011 and 1105 alleles in patients with different grades of hypertension

DRB1* *03011*	*Hypertension*			*Total*
	Severe	Non-severe	Absent	
+	11	4	0	15
-	10	3	4	17
Total	21	7	4	32

P= 0.13

DRB1* *1105*	*Hypertension*			*Total*
	Severe	Non-severe	Absent	
+	6	1	1	8
-	15	6	3	24
Total	21	7	4	32

P= 0.75

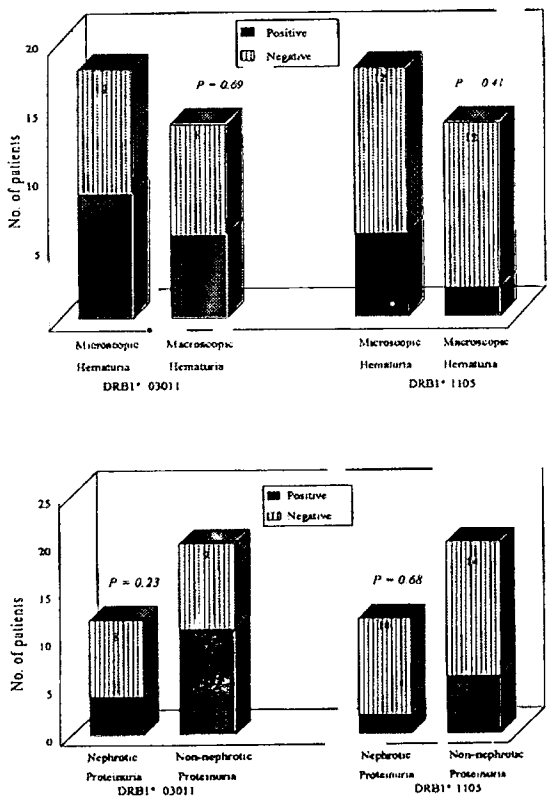

Fig. 1. DRB1* 03011 & 1105 frequencies in patients with different grades of proteinuria and hematuria

Discussion

In this study, we demonstrated a significant association, tested by RR, of PSAGN with DRB1* 03011 and 1105 alleles. The insignificantly higher frequency of DRB1* 1105 when P value was corrected for the number of alleles tested may be due to the small number of patients included in the study.

Well in 1812 [15] was the first one who suspected the familial predisposition to PSAGN. He observed that the siblings of the child who developed nephritis after scarlet fever were much more likely to develop nephritis than the siblings of children who developed scarlet fever without nephritis. Sasazuki et al. in 1983 [16] assumed that patients with PSAGN are genetically programmed to respond abnormally to streptococcal infection. Recently it is proposed that streptococcal M proteins and pyrogenic exotoxins can act as superantigens that cause a marked expansion of T cells expressing specific T-cell receptor β -chain variable gene segments [17-19]. It is generally agreed that HLA II alleles play a central role in recognition of antigens by T-cells [20]. The association of PSAGN and DRB1* 03011 and 1105 alleles, may be due to the direct influence of these alleles on the immune system predisposing children to PSAGN. However, this association may result from the effect of non-HLA genes in linkage disequilibrium with one or more of these alleles [21].

Many studies were done on the association of PSAGN with HLA class II. The results, however, do not point to a universal marker of the disease. Read et al. showed no HLA association of the disease in East Indian patients [3]. Significant association with DR4 in had been reported from Venezuela [4]. Sasazuki et al. reported that Japanese patients with PSAGN have increased frequency of DR w13-Dw19 haplotype [5]. This haplotype corresponds to HLA-DRB1* 1302-DQA1* 0102-DQB1* 0604 [22]. However, Mori et al. [23] reported an association of DPB1* 0501 with patients with PSAGN in Japan with no differences in the frequencies of DRB1 or DQB1 between patients and controls. This discrepancy may be explained by the difference in HLA distribution among different population.

The absence of difference in the frequency of DRB1* 03011 and 1105 between patients with different grades of the disease points to the insignificant role of these alleles in affecting the severity of the disease. Reported association of HLA class II alleles with the severity of the disease had been reported with haplotypes rather than with alleles, there are even fewer data from experimental studies to help interpretation of these results [24]. We conclude that DRB1* 03011 and/or 1105 might be a restricting element in the presentation of disease-causing streptococcal antigenic peptide. However they do not affect the severity of the disease.

References

1. Glassock RJ, Brenner CM: In Brenner BM (Ed). The Kidney. Saunders, Philadelphia, 1973; 961.
2. Braun DG, Schalch W, Schmid I: In Read SE, Zabriskie JB (Eds) Streptococcal Diseases and the Immune Response, * Academic, New York, 1980; 317.
3. Read SE, Reid H, Poon King T, Fischetti VA, Zabriskie JB, Rapaport FT: HLA and predisposition to the non-suppurative sequalae of group A streptococcal infections. Transplant Proc, 1977; 9:543-6.
4. Layrisse Z, Rodriguez-Itrube B, Garcia-Ramirez R, Rodriguez A, Tiwari J: Family studies of the HLA system in acute poststerptococcal glomerulonephritis. Hum Immunol, 1983; 7:177-85.
5. Sasazuki T, Hayase R, Iwamoto I, Tsuchida H: HLA and acute streptococcal glomerulonephritis. N. Engl J. Med, 1979; 301:1184-5.
6. Unanue ER, Allen PM: The basis for the immunoregulatory role and other accessory cells. Science, 1987; 235:551-557.
7. Marsh SG: Nomenclature for factors of HLA system, update January/February 1997. WHO Nomenclature Committee for Factors of HLA System. Hum Immunol, 1997; 53: 224.
8. Bodmer WF: HLA: What's the name? A commentary on HLA nomenclature development over the years. Tissue Antigens, 1997; 49:293-296.
9. Report of second task force on blood pressure control in children-1987: Report of second task force on blood pressure control in children. Pediatrics, 1987; 79:1-25.
10. Buyse I, Decorte R, Beans M, et al: Rapid DNA typing of class II HLA antigens using the polymerase chain reaction and reverse dot blot hybridization. Tissue Antigens, 1993; 41:1-14.
11. Thonnard J, Deldime F, Heusterpreute M, et al: HLA class II genotyping: two assay systems compared. Clin Chem, 1995; 41:553-556.

12. Vaughan RW: PCR-SSO typing for HLA-DRB alleles. Eur J Immunogen, 1991; 18-69-80.
13. Emery AEH: In Emery AEH (Ed) Methodology in medical genetics, 1st edn. Churchill Livingstone, Edinburgh. 1976; 98.
14. Bengtsson Bo, Thomoson G: Measuring the strength of association between HLA antigens and diseases. Tissue Antigens, 1981;18:356-363.
15. Well WC: Observation on dropsy which succeeds scarlet fever. Trans Soc Improve Med Chir Know. 1812; 13:13:167-168
16. Sasazuki T, Nishimura Y, Muto M, Ohta N.: Linked genes controlling the immune response and disease susceptibility. Immunol Rev. 1983; 0:51-75.
17. Tomat M, Kotb M, Majumdar G, Beachey EH: Super-antigencity of streptococcal M protein. J. Exp Med. 1990; 172: 359-362.
18. Abc J, Forrester J, Nakahara T, Lafferty JA, Kotzin BL, Leung DY: Selective stimulation of human T cells with streptococcal erythrogenic toxins A and B. J Immunol 1991, 146: 3747-3750.
19. Kotzin BL, Leung DY, Kappler J, Marrack P: superantigens and their potential role in human disease. Adv immunol 1993: 54: 99-166.
20. Yoshizawa N, Treser G, McClung JA. Sagel I, Takahashi K Circulating immune complexes in patients with uncomplicated group A streptococcal pharyngitis and patients with acute post streptococcal glomerulonephritis. Am J. Nephrol 1983; 3: 23-29.
21. Tomlinson IP, Bodmer WF: The HLA system and the analysis of multifactorial disease. Trends Genet, 1995; 11:493-498.
22. Imanishi T, Akaza T. Kimura A, Tokunaga K, Gojobori T. Allele frequencies and haplotype frequencies for HLA and complement loci in various ethnic groups. In: Tsji K, Aizawa M, Sasazuki T (Eds). HLA 1991. Oxford University Press, Oxford, 1992; 1: 1065-220.
23. Mori K, Sasazuki T. Kimura A and Ito YP: HLA-DP antigens and post-streptococcal acute glomerulonephritis. Acta Pediatr 1996; 85: 916-918
24. Rees AG. Immunogenetics of renal disease. In: Neilson Eg, Couser WG (Eds). Immunologic renal disease. Lippincott-Raven Press. Philadelphia. 1997; 99-120.