Effects of HLA-DR, DQ Genes and Cytotoxic T Lymphocyte Antigen-4 Gene Polymorphisms on the Development of Type 1 Diabetes in Korean Children

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Introduction

Type 1 diabetes is a multifactorial autoimmune disease, which is characterized by T cell mediated destruction of the insulin secreting β-cells in the pancreas. HLA system has been consistently implicated as a major genetic risk factor in type 1 diabetes, but population studies have shown that HLA associations may vary depending on geographic and ethnic origin. Recent studies have demonstrated associations between cytotoxic T lymphocyte antigen-4 (CTLA-4) gene polymorphisms and some autoimmune diseases. The aim of the present study was to assess the effect of HLA class II alleles on susceptibility to type 1 diabetes and to evaluate the association of CTLA4 gene polymorphisms with type 1 diabetes in Korean children.

Subjects and Methods

A total 188 Korean subjects with childhood-onset type 1 diabetes (96 females and 92 males) were studied along with 200 healthy control subjects. HLA-DRB1 and DQB1 alleles were determined by PCR-single strand conformational polymorphism and PCR-restriction fragment length polymorphism (RFLP) methods. The CTLA4 exon 1 polymorphism at position 49 and CTLA4 promoter -318 polymorphism were analyzed by a PCR-RFLP method.

Results

Predisposition to type 1 diabetes was associated with the DRB1*0301-DQB1*0201, DRB1*0405-DQB1*0401, and DRB1*09012-DQB1*03032 haplotypes, whereas the DRB1*1202-DQB1*0301 and DRB1*1501-DQB1*0602 haplotypes were protective haplotypes (Table 1). The genotypes and allele frequencies of CTLA4 exon 1 position 49 A/G and promoter -318 C/T polymorphisms did not differ between the patients and the control subjects (Table 2, 3). These polymorphisms were not associated with clinical characteristics and the development of autoimmune thyroid disease in type 1 diabetic patients. The frequency of AA genotype of CTLA4 exon 1 polymorphism was significantly higher in the patients without both HLA-DRB1*0301 and DRB1*09012 alleles than in the control subjects (36.0% *vs* 14.4%; *P*<0.05) (Table 5). The frequency of A allele was significantly higher in the patients without two alleles among susceptible HLA-DRB1 alleles, which are DRB1*0301, *0405 and *09012, than in the control subjects (*P*<0.05) (Table 5).

Table 1. Expected HLA DRB1-DQB1 Haplotype Frequencies in the Patients with Type 1 Diabetes and the Controls Subjects

DRB1-DQB1	Patients (n=116) N (%)	Controls (n=200) N (%)	*P*	OR	DRB1-DQB1	Patients (n=116) N (%)	Controls (n=200) N (%)	*P*	OR
0101-0501	15 (13)	28 (14)			1001-0501	2 (2)	9 (5)		
0301-0201	35 (30)	7 (4)	<0.001	11.91	1101-0301	2 (2)	13 (7)		
0401-03032	0	1 (1)			1201-0301	3 (3)	12 (6)		
0402-03032	1 (1)	0			1201-0302	1 (1)	1 (1)		
0403-0302	7 (6)	10 (5)			1201-03032	2 (2)	5 (3)		
0404-0302	1 (1)	1 (1)			1202-0301	0	18 (9)	<0.01	0.04
0404-0402	0	3 (2)			1301-0603	0	6 (3)		
0405-0302	2 (2)	0			1302-0604	9 (7)	25 (13)		
0405-03032	0	3 (2)			1302-0609	2 (2)	8 (4)		
0405-0401	43 (37)	30 (15)	<0.001	3.34	1401-0301	0	1 (1)		
0406-0301	0	1 (1)			1401-0502	1 (1)	6 (3)		
0406-0302	3 (3)	20 (10)			1401-05031	0	5 (3)		
0407-0302	2 (2)	1 (1)			1402-0301	0	2 (1)		
0407-0401	0	1 (1)			1403-0301	0	5 (3)		
0410-0402	1 (1)	4 (2)			1405-03032	0	1 (1)		
0701-0202	14 (12)	25 (13)			1405-05031	0	14 (7)		
0701-03032	0	2 (1)			1407-05031	0	1 (1)		
0802-0302	10 (9)	5 (3)			1501-0301	1 (1)	0		
0802-0402	0	6 (3)			1501-03032	1 (1)	0		
0803-0301	0	4 (2)			1501-0602	4 (3)	33 (17)	<0.05	0.18
0803-0302	0	1 (1)			1501-0603	0	1 (1)		
0803-0601	3 (3)	25 (13)			1502-0501	0	2 (1)		
09012-03032	51 (44)	33 (17)	<0.001	3.97	1502-0601	1 (1)	7 (4)		

Table 2. Distribution of CTLA4 Exon 1 Polymorphism in the Patients with Type 1 Diabetes and the Control Subjects

		Genotype (%)			Allele (%)	
	n	AA	AG	GG	A	G
Type 1 Diabetes	188	13.6	33.0	53.4	30.1	69.9
with AITD	32	16.1	32.3	51.6	32.3	67.7
DKA at diagnosis	43	14.0	27.9	58.1	27.9	72.1
SMR at diagnosis						
prepubertal	139	15.8	32.4	51.8	32.0	68.0
in puberty	37	7.9	34.2	57.9	23.0	77.0
Controls	91	14.4	34.4	51.1	31.7	68.3

AITD, autoimmune thyroid disease; DKA, diabetic ketoacidosis; SMR, sexual maturity rating

Table 3. Distribution of CTLA4 Promoter -318 Polymorphism in the Patients with Type 1 Diabetes and the Control Subjects

		Genotype (%)			Allele (%)	
	n	CC	CT	TT	C	T
Type 1 Diabetes	188	78.2	20.7	1.1	88.6	11.4
with AITD	31	80.6	19.4	0	90.3	9.7
DKA at diagnosis	43	72.3	25.5	2.1	85.1	14.9
SMR at diagnosis						
prepubertal	147	76.9	22.4	0.7	88.1	11.9
in puberty	41	82.9	14.6	2.4	90.2	9.8
Controls	91	79.1	18.7	2.2	88.5	11.5

AITD, autoimmune thyroid disease; DKA, diabetic ketoacidosis; SMR, sexual maturity rating

Table 4. Distribution of CTLA4 Exon 1 Polymorphism in the Patients with Type 1 Diabetes According to HLA-DRB1 Alleles

		Genotype (%)			Allele (%)	
	n	AA	AG	GG	A	G
DRB1*0301 (+)	24	16.7	33.3	50.0	33.3	66.7
(-)	55	25.5	36.4	38.2	43.6[a]	56.4
DRB1*0405 (+)	29	31.0	20.7	48.3	41.4	58.6
(-)	50	18.0	44.0	38.0	40.0	60.0
DRB1*09012(+)	35	17.1	40.0	42.9	37.1	62.9
(-)	43	27.9	30.2	42.5	42.5	57.5
Controls	90	14.4	34.4	51.1	31.7	68.3

[a]*P*<0.05, OR=1.67 (1.02-2.73) *vs* controls

Table 5. Distribution of CTLA4 Exon 1 Polymorphism in Type 1 Diabetic Patients without Two Susceptible HLA-DRB1 Alleles

		Genotype (%)			Allele (%)	
	n	AA	AG	GG	A	G
DRB1*0301(-)/0405(-)	33	24.2	42.4	33.3	45.5[b]	54.5
DRB1*0301(-)/09012(-)	25	36.0[a]	32.0	32.0	52.0[c]	48.0
DRB1*0405(-)/09012(-)	12	25.0	58.3	16.7	54.2[d]	45.8
Controls	91	14.4	34.4	51.1	31.7	68.3

[a]*P*<0.05, OR=3.33 (CI 1.21-9.11) *vs* controls
[b]*P*<0.05, OR=1.80 (CI 1.01-3.21) *vs* controls
[c]*P*<0.01, OR=2.34 (CI 1.24-4.42) *vs* controls
[d]*P*<0.05, OR=2.55 (CI 1.08-6.02) *vs* controls

Table 6. Distribution of CTLA4 Promoter -318 Polymorphism in the Patients with Type 1 Diabetes According to HLA-DRB1 Alleles

		Genotype (%)			Allele (%)	
	n	CC	CT	TT	C	T
DRB1*0301 (+)	24	87.5	12.5	0	93.8	6.3
(-)	56	78.2	21.8	0	89.1	10.9
DRB1*0405 (+)	31	86.2	13.8	0	93.1	6.9
(-)	49	78.0	22.0	0	89.0	11.0
DRB1*09012(+)	35	82.9	17.1	0	91.4	8.6
(-)	45	79.5	20.5	0	89.8	10.2
Controls	91	79.1	18.7	2.2	88.5	11.5

Conclusions

These results suggest that HLA-DRB1 and DQB1 genes are strong genetic determinants for type 1 diabetes. CTLA4-mediated susceptibility effect on the development of type 1 diabetes might be significant in the absence of susceptible HLA class II alleles.