diagnose hemorrhagic shock following injury, which is accompanied with hypotension and tachycardia (6, 7). In the present study, women who were to give birth in winter had a high SI in their first or second trimester. Women who were to give birth in winter spent their first trimester or second trimester during the summer season. The first trimester SI of the women who were to give birth in winter was 0.72–0.77 bpm/mmHg, while the first trimester SI of the women who were to give birth in other seasons was 0.66–0.70 bpm/mmHg. Birkhahn et al. reported that acute blood loss of 450 mL significantly increased the SI from 0.61 to 0.65 bpm/mmHg (7). SI might also represent hypovolemia. Women who are to give birth in winter might have hypovolemia in their first to second trimester. Similarly, women who are to give birth in autumn might have hypovolemia in their last trimester.

Plasma volume in pregnancies complicated by preeclampsia is reported to be significantly lower than in normal pregnancies in the first trimester (2). In this study, plasma volume is reported to be the first parameter to show significant intergroup difference among the parameters of progesterone, aldosterone, estradiol and their combination. Although further studies are necessary to investigate which factors changed before and after hypovolemia, there might be

some association between hypovolemia in the first trimester in summer and the incidence of preeclampsia in winter. SI might be a good marker of dehydration in summer, since a similar trend was observed for HR (Fig. 2).

Limitations

There are some limitations in this study. First, SI seems to be a good way to identify hypovolemia within one subject; however, it is impossible to compare SIs among individuals because the SI is low with high BP. Another method may be necessary to identify hypovolemia. There are no previous reports showing that SI reflects chronic hypovolemia, in the same way as acute hypovolemia. Further study is needed to evaluate the amplitude of hypovolemia in chronic conditions. Second, in our study, we did not perform echocardiography or electrocardiography; therefore, we cannot evaluate the real clinical meaning of the DP using such physiological examinations. Another approach might be necessary to evaluate the real clinical meaning of DP. Third, these data are limited to normotensive pregnant women, because we did not perform a similar analysis in preeclamptic women, since few subjects developed preeclampsia. Serial changes of indirect indices might be modified by

hospitalization, medication, and termination in subjects with preeclampsia.

Conclusion

This study collected daily serial hemodynamic data during pregnancy using home BP monitoring. DP increased gradually as gestational age increased, and the effect of seasonality and expected date of birth on DP was less marked than that of gestational age. SI might be useful for identifying hypovolemia within individuals. Such data might be useful for examining hemodynamic changes during normal pregnancy, as well as identifying hemodynamic changes during abnormal pregnancy.

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Figure Legends

Figure 1: Systolic blood pressure (SBP, (a)), heart rate (HR, (b)), double product (DP (c)), and shock index (SI (d)) values and their 95% confidence intervals for each week of gestational age, calculated on the basis of a mixed linear model.

Figure 2: Systolic blood pressure (SBP, (a)), heart rate (HR, (b)), double product (DP (c)), and shock index (SI (d)) values and their 95% confidence intervals for each week for a year, calculated on the basis of a mixed linear model without adjusting for seasonal variation.

Figure 3: Systolic blood pressure (SBP, (a)), heart rate (HR, (b)), double product (DP (c)), and shock index (SI (d)) values for the combination of gestational age and expected date of birth, calculated on the basis of a mixed linear model. The horizontal axis shows gestational age, and the vertical axis shows the expected date of birth.

Table 1. Associations between hemodynamic parameters and daily minimum outside temperature in summer and in other seasons.

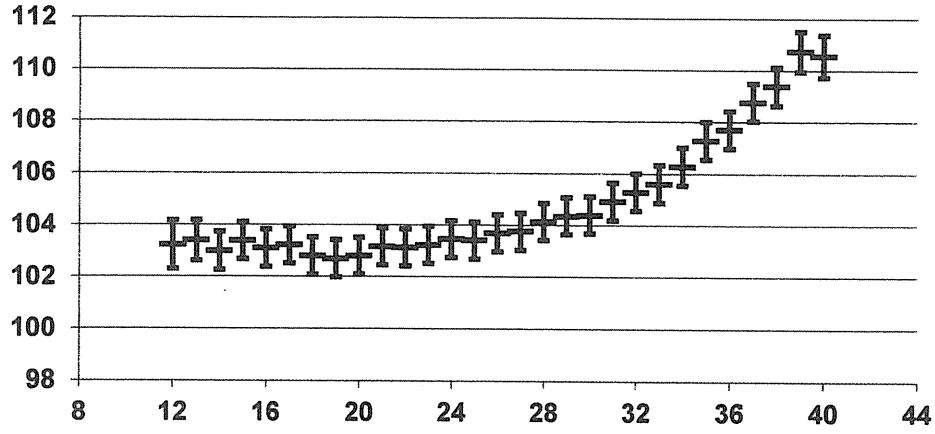
	Summer (from June to September)			Other seasons (from October to May)			Interaction*
	β	SE	p	β	SE	p	p
SBP (mmHg)	−0.3055	0.0049	<0.0001	−0.1999	0.0074	<0.0001	<0.0001
HR (bpm)	0.0095	0.0052	<0.0001	−0.0560	0.0080	<0.0001	<0.0001
DP (10^2·mmHg·bpm)	-0.2172	0.0069	<0.0001	−0.2051	0.0104	<0.0001	<0.0001
SI (10^{-2}·bpm/mmHg)	0.2235	0.0058	<0.0001	0.0811	0.0088	<0.0001	<0.0001

SBP: systolic blood pressure, HR: heart rate, DP: double product, SI: shock index.

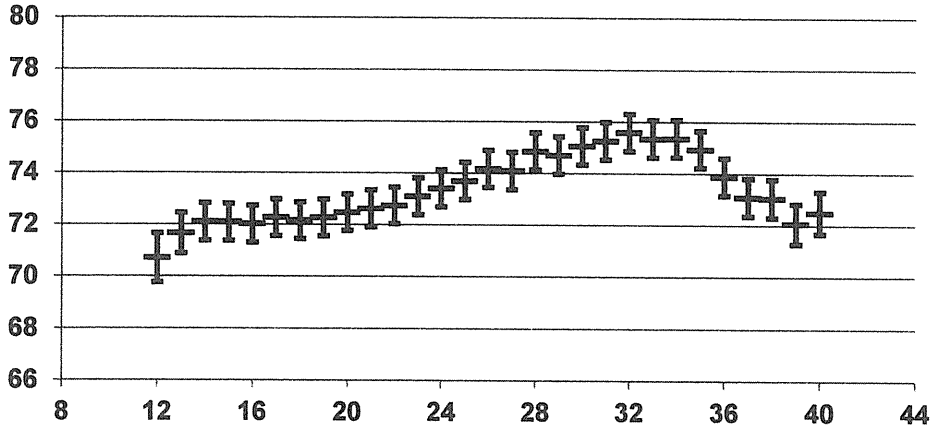
*: Interaction between daily minimum outside temperature and seasonality and hemodynamic parameters.

Figure 1

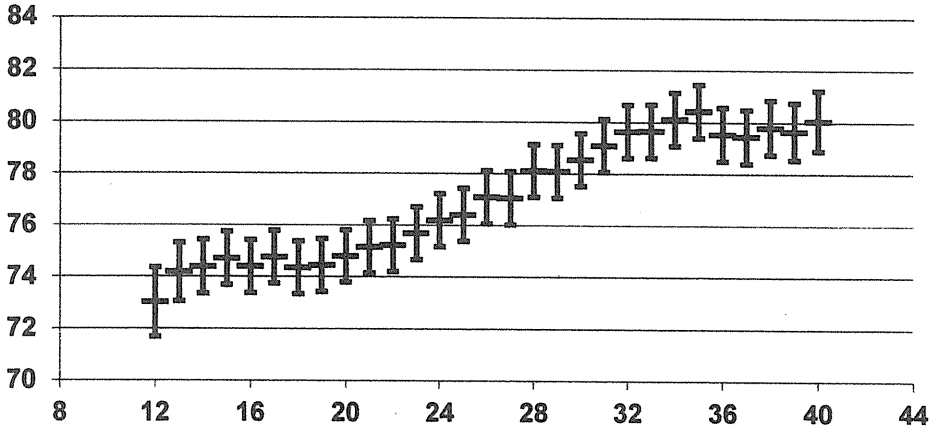
a) SBP (mmHg)



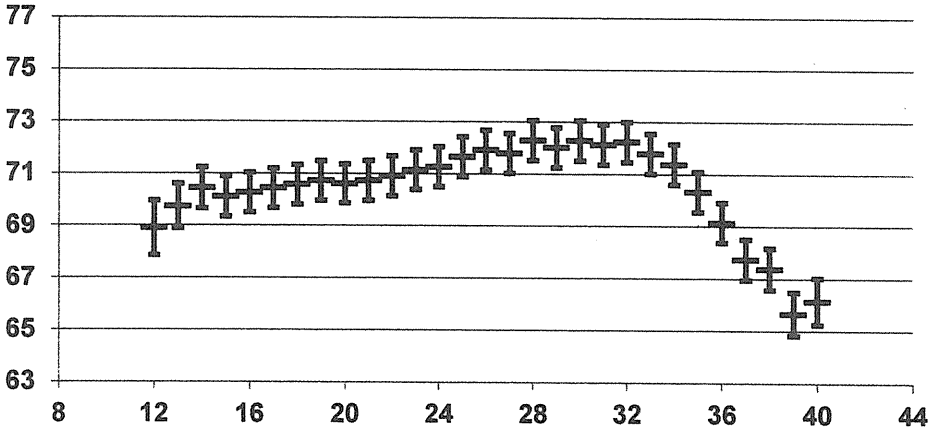
b) HR (bpm)



c) DP(10^2•mmHg•bpm)



d) SI(10^{-2}•bpm/mmHg)

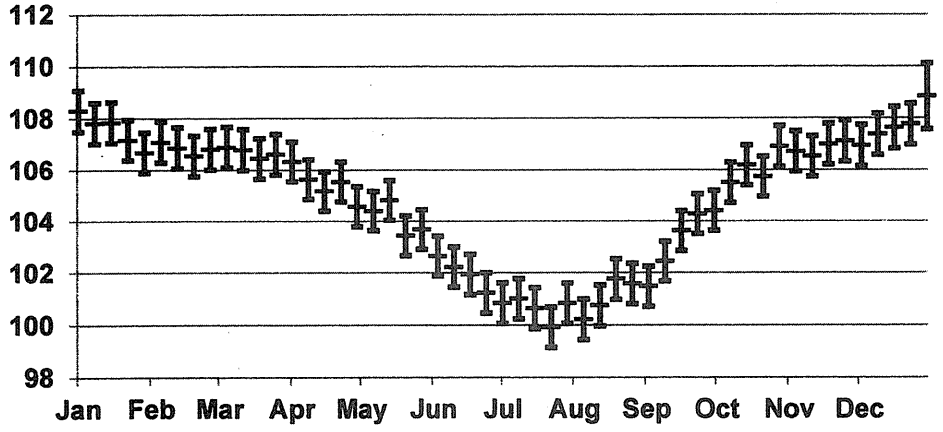


Gestational Age (weeks)

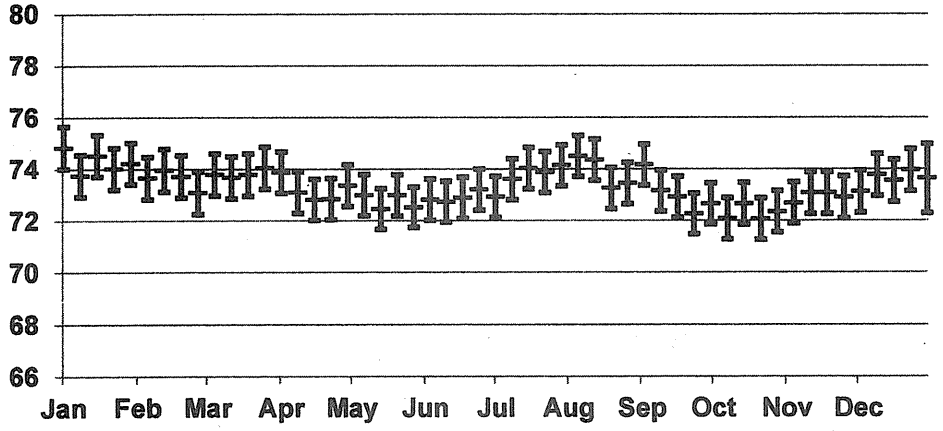
25

Figure 2

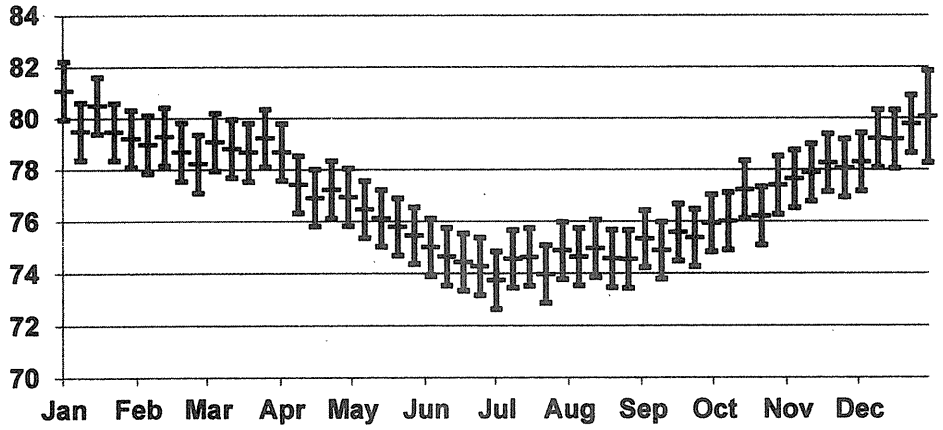
a) SBP (mmHg)



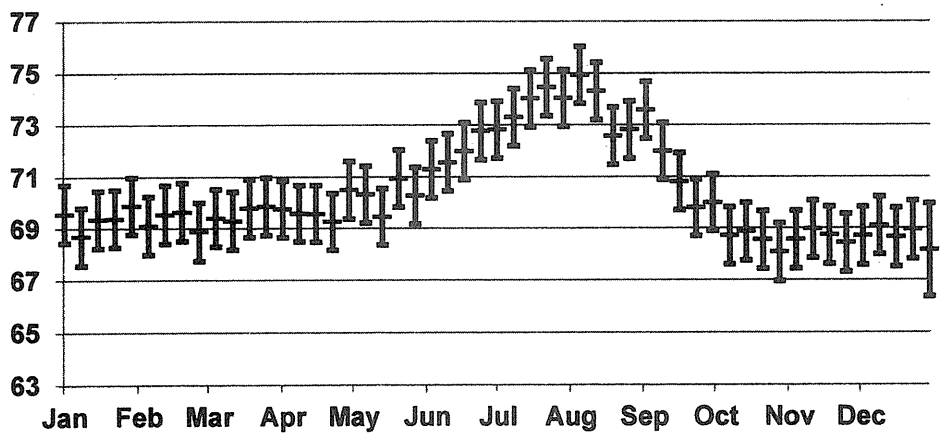
b) HR (bpm)



c) DP(10^2·mmHg·bpm)

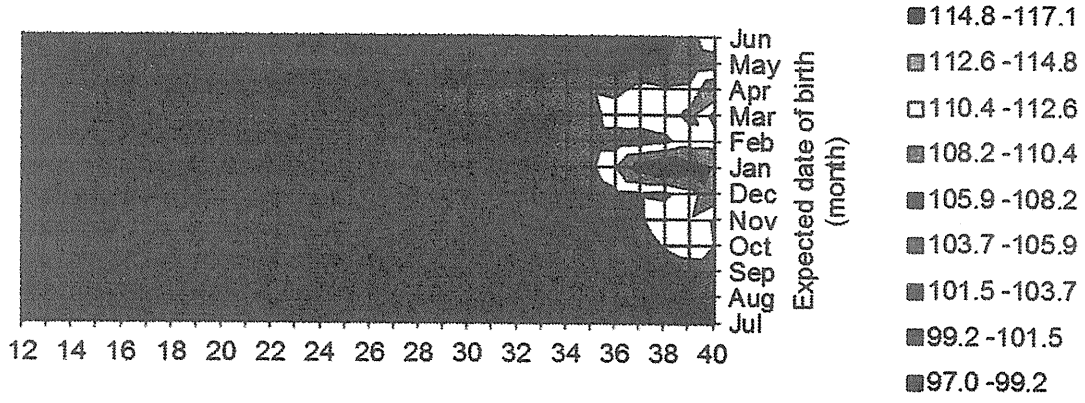


d) SI(10^{-2}·bpm/mmHg)

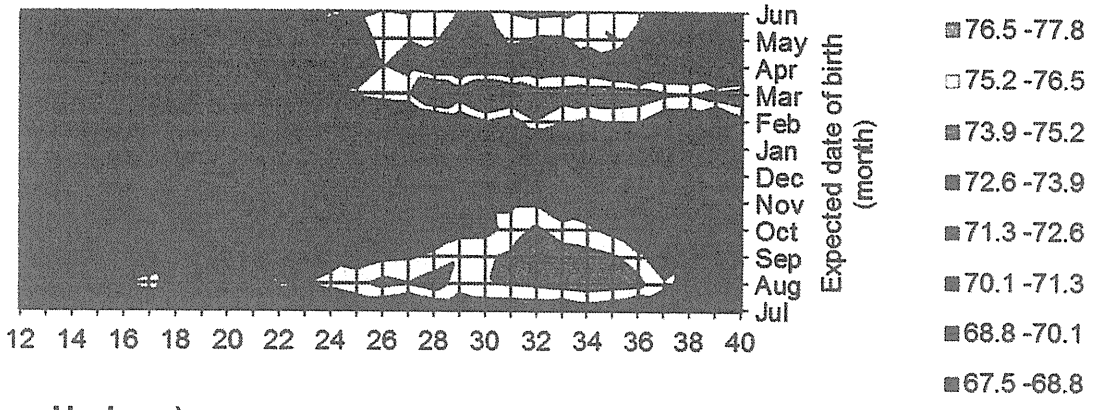


26

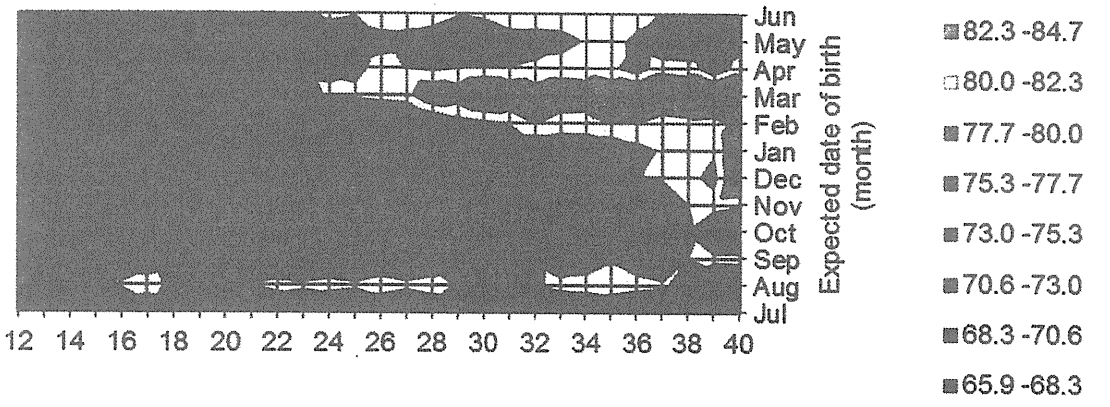
Figure 3
a) SBP (mmHg)



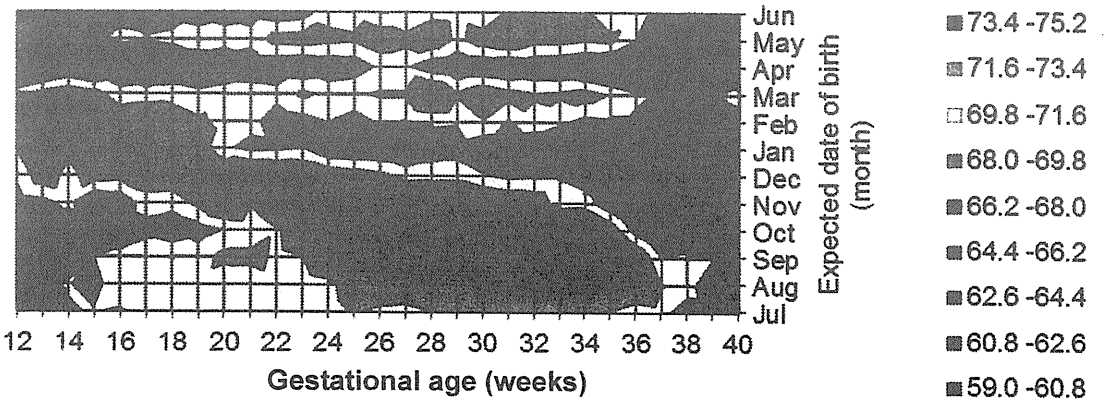
b) HR (bpm)



c) DP(10^2•mmHg•bpm)



d) SI(10^{-2}•bpm/mmHg)



Pediatric Diabetes

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Pediatric Diabetes

Original Article

HLA-class II and class I genotypes among Japanese children with Type 1A diabetes and their families

Sugihara S, Ogata T, Kawamura T, Urakami T, Takemoto K, Kikuchi N, Takubo N, Tsubouchi K, Horikawa R, Kobayashi K, Kasahara Y, Kikuchi T, Koike A, Mochizuki T, Minamitani K, Takaya R, Mochizuki H, Nishii A, Yokota I, Kizaki Z, Mori T, Shimura N, Mukai T, Matsuura N, Fujisawa T, Ihara K, Kosaka K, Kizu R, Takahashi T, Matsuo S, Hanaki K, Igarashi Y, Sasaki G, Soneda S, Teno S, Kanzaki S, Saji H, Tokunaga K, Amemiya S and The Japanese Study Group of Insulin Therapy for Childhood and Adolescent Diabetes (JSGIT). HLA-class II and class I genotypes among Japanese children with Type 1A diabetes and their families.
Pediatric Diabetes 2012: 13: 33–44.

Objective: To determine the HLA-DRB1, DQB1, DPB1, A, C, and B genotypes among Japanese children with autoimmune type 1 diabetes.
Methods: Four hundred and thirty patients who were GADAb and/or IA-2Ab-positive (Type 1A) were recruited from 37 medical centers as part of a nationwide multicenter collaborative study. DNA samples from 83 siblings of the children with Type 1A diabetes and 149 parent–child trios were also analyzed. A case-control study and a transmission disequilibrium test (TDT) were then performed.
Results: The susceptible and protective DRB1 and DQB1 alleles and haplotypes were confirmed. DPB1 alleles unique to the Japanese population and those common to multiple ethnic groups were also present. A linkage disequilibrium (LD) analysis showed both susceptible and protective haplotypes. The TDT did not reveal any alleles that were transmitted preferentially from the mother or father to children with Type 1A. Homozygosity for DRB1*09:01-DQB1*03:03 and heterozygosity for DRB1*04:05-DQB1*04:01 and DRB1*08:02-DQB1*03:02 were associated with an extremely high risk of Type 1A. A comparison of children with Type 1A and their parents and siblings suggested a dose effect of susceptible DRB1-DQB1 haplotypes and an effect of protective alleles on immunological pathogenesis. DRB1*09:01 appeared to be strongly associated with an early onset in preschool children with Type 1A diabetes.
Conclusions: This study demonstrated the characteristic association of HLA-class II and class I genes with Type 1A diabetes among Japanese children. A TDT did not reveal the genomic imprinting of HLA-class II and class I genes in Type 1A diabetes.

Genetic and environmental factors are thought to be responsible for differences in the incidence of type 1 diabetes among different ethnic groups. The contribution of the HLA-DRB1, DQA1, and DQB1 genes to susceptibility to autoimmune type 1 diabetes (Type 1A) has been well described (1, 2). Several genome scans for linkage to type 1 diabetes have been performed, and these studies have indicated that a gene or genes in the HLA region (insulin-dependent diabetes mellitus 1) at 6p21 has or have

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the strongest impact on disease risk (2, 3). In addition, the independent effects of HLA-DPB1, A, and B have also been demonstrated (4, 5).

The incidence of childhood-onset type 1 diabetes mellitus in Japan is very low (1.4–2.2/100 000 individuals per year) compared with Caucasian populations, especially in Europe (Sardinia, Finland, Sweden, and the UK) and Canada (20/100 000 per year) (6). The risk for siblings of individuals with type 1 diabetes is similar between Caucasians (about 6%) and Japanese (3.8%) (7, 8). These results suggest the existence of both a different set of immunogenetic mechanisms in Japanese patients with type 1 diabetes and a common pathogenesis with Caucasian patients.

The genetic effects of HLA-DRB1 and DQB1 in Japanese patients with type 1 diabetes reportedly differ from those in Caucasian patients (9–15). In Caucasian populations, a predisposition to type 1 diabetes is mostly associated with the DRB1*03:01-DQA1*05:01-DQB1*02:01 and/or DRB1*04:01-DQA1*03:01-DQB1*03:02 haplotypes, whereas the DRB1*15:01-DQB1*06:02 haplotype confers strong protection against the disease. In the Japanese population, three characteristic haplotypes confer susceptibility to type 1 diabetes: DRB1*04:05-DQB1*04:01, DRB1*08:02-DQB1*03:02, and DRB1*09:01-DQB1*03:03. Furthermore, two haplotypes confer protection: DRB1*15:01-DQB1*06:02 (which is common among Caucasians), and DRB1*15:02-DQB1*06:01 (which is characteristic of the Japanese population) (11–15).

HLA-DPB1 alleles are not generally recognized as major contributors to type 1 diabetes. However, an increased risk associated with allele DPB1*02:02 and *03:01 and a decreased risk associated with allele *04:02 have been reported in a number of ethnic groups (4, 5, 16–19). The association of DPB1*02:01 with Japanese childhood-onset type 1 diabetes has been reported by Nishimaki et al. (20), but the number of subjects in this study was relatively small.

This study is the first nationwide multicenter collaborative study for genetic factors in Japanese children with type 1 diabetes and their families. The objective of this study was to determine the genetic characteristics of both HLA-class II (DRB1, DQB1, and DPB1), and class I (A, C, and B) genotypes among Japanese children with Type 1A diabetes and to compare these characteristics with both control data and data obtained from the parents and siblings of the children with Type 1A diabetes. We also studied the diabetes-associated allelic transmission rates from mothers and fathers to children with Type 1A diabetes in the Japanese population.

Methods

Subjects

We recruited 497 Japanese children with type 1 diabetes from 37 medical centers throughout Japan between February 2008 and February 2009. The patients were divided into two groups: Type 1A (GADAb and/or IA-2Ab-positive at diagnosis and/or at registration in this study) and Type 1B (GADAb and IA-2Ab-negative). Type 1A accounted for 430 patients (158 boys and 272 girls) who were 0.8–16.4 years old (mean ± SD, 7.6 ± 3.7 years) at the time of diagnosis. Type 1B accounted for 67 patients (28 boys and 39 girls) who were 0.1–15.1 years old (6.2 ± 4.4 years) at the time of diagnosis. In this study, we focused on children with Type 1A diabetes. Type 1B diabetes may have heterogeneous pathogenetic mechanisms, and some cases of Type 1B have been shown to have a particular monogenic cause, such as mutations in the insulin gene (*INS*), *KCNJ11*, or *ABCC8*. Furthermore, the number of subjects with Type 1B diabetes was too small to obtain a sufficient power in the case-control study.

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Clinical data for all the type 1 diabetes children were obtained. The diagnosis of type 1 diabetes was based on both clinical features and laboratory data. All the patients with Type 1A diabetes were ketosis-prone, lacked endogenous insulin secretion, and required insulin injections at the time of diagnosis based on the 1999 Japan Diabetes Society criteria. The HbA1c levels at the time of diagnosis were $11.9 \pm 2.6\%$ among the patients with Type 1A diabetes. The insulin dose at the time of study registration was 1.1 ± 0.3 units/kg/day among the patients with Type 1A diabetes. Eighty-three siblings of 66 children with Type 1A diabetes and 148 father and mother pairs of 149 children with Type 1A diabetes (149 parent–child trios) were recruited. The control data for the HLA allele and haplotype frequencies were based on previously reported data for 1216 subjects in a general Japanese population (21) and a study of 159 families with 561 subjects (22).

This study was approved by the institutional ethics review board of the Tokyo Women's Medical University, the National Research Institute for Child Health and Development, and each of the clinics or hospitals affiliated with a study collaborator. Written informed consent was obtained from the parents or guardians and/or the participants.

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Key words: genetic factors – HLA – Japanese children – type 1 diabetes

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HLA typing

Genomic DNA was extracted from whole blood samples. HLA typing was performed using a Luminex Multi-Analyte Profiling system with a WAKFlow HLA typing Kit (Wakunaga, Hiroshima, Japan), as described elsewhere (23). Briefly, highly polymorphic exons 2 and 3 of the HLA-A, -B, and -C genes and exon 2 of the HLA-DRB1, -DQB1, and -DPB1 genes were amplified using the primer pairs included with the kit. Each polymerase chain reaction product was hybridized using sequence-specific oligonucleotide probes that were complementary to the allele-specific sequences.

Statistical analysis

All the statistical analyses were performed using the R statistical environment, version 2.9.1 (http://www.r-project.org/). The Fisher exact test was applied to a two-by-two contingency table, and the corrected p values (Pc), equivalent to the p values multiplied by the number of comparisons for each locus or haplotype, were determined. A Pc value <0.05 was considered statistically significant.

The study had a sufficient power (more than 0.98) to detect an odds ratio (OR) $= 2.0$ for an allele frequency of 0.1 in the case-control study comparing DRB1, DQB1, DPB1, A, C, and B between the children with Type 1A diabetes ($n = 430$) and the control data.

The frequency of HLA haplotypes was estimated using the maximum likelihood method (24) or the PHASE program (25). Relative linkage disequilibrium (RD) was calculated as the linkage disequilibrium (LD)/|Dmax| for the relative assessment of LD (22). |Dmax| was the absolute value of the maximum LD for the haplotype.

Results

Association of HLA-DRB1, DQB1, and DPB1 with Type 1A diabetes

In the case-control study, the susceptible alleles associated with Type 1A diabetes in Japanese children were DRB1*09:01 ($Pc < 10^{-29}$; OR, 3.00), DRB1*04:05 ($Pc < 10^{-20}$; OR, 2.60), DRB1*08:02 ($Pc < 10^{-12}$; OR, 3.11), DQB1*03:03 ($Pc < 10^{-26}$; OR, 2.80), DQB1*04:01 ($Pc < 10^{-16}$; OR, 2.32), DQB1*03:02 ($Pc < 10^{-12}$; OR, 2.34), DPB1*02:01 ($Pc < 10^{-2}$; OR, 1.49), and DPB1*03:01 ($Pc < 0.05$; OR, 1.92). The protective alleles were DRB1*15:02 ($Pc < 10^{-21}$; OR, 0.09), DRB1*15:01 ($Pc < 10^{-16}$; OR, 0.06), DRB1*08:03 ($Pc < 10^{-14}$; OR, 0.14), DRB1*04:06 ($Pc < 10^{-3}$; OR, 0.23), DQB1*06:01 ($Pc < 10^{-36}$; OR, 0.11), DQB1*06:02 ($Pc < 10^{-19}$; OR, 0.00), DQB1*03:01 ($Pc < 10^{-11}$; OR, 0.29), DPB1*09:01 ($Pc < 10^{-8}$; OR, 0.25), and DPB1*04:02 ($Pc < 10^{-2}$; OR, 0.57) (Table 1).

The susceptible HLA-DRB1-DQB1 haplotypes associated with Type 1A diabetes in Japanese children were DRB1*09:01-DQB1*03:03 ($Pc < 10^{-20}$; OR, 3.05), DRB1*04:05-DQB1*04:01 ($Pc < 10^{-10}$; OR, 2.33), DRB1*08:02-DQB1*03:02 ($Pc < 10^{-11}$; OR, 5.41), and DRB1*04:05-DQB1*03:02 ($Pc < 10^{-11}$). The protective HLA-DRB1-DQB1 haplotypes were

Table 1. HLA-DRB1, DQB1, and DPB1 allele frequencies among Japanese children with Type 1A diabetes

		Type 1A		Control		Type 1A vs. Control		
HLA	Allele	n = 860	%	n	%	Pc	OR	(95% CI)
DRB1	*04:05	244	28.37	322	13.26	$<10^{-20}$	2.60	(2.15–3.14)
	*08:02	103	11.98	102	4.18	$<10^{-12}$	3.11	(2.34–4.14)
	*09:01	283	32.91	342	14.08	$<10^{-29}$	3.00	(2.50–3.60)
	*04:06	6	0.70	73	3.00	$<10^{-3}$	0.23	(0.10–0.52)
	*08:03	11	1.28	202	8.29	$<10^{-14}$	0.14	(0.08–0.26)
	*15:01	4	0.47	173	7.11	$<10^{-16}$	0.06	(0.02–0.16)
	*15:02	9	1.05	246	10.13	$<10^{-21}$	0.09	(0.05–0.18)
	*13:02	39	4.53	166	6.83	NS		
	Others	161	18.72	806	33.14			
DQB1	*03:02	167	19.42	227	9.32	$<10^{-12}$	2.34	(1.88–2.91)
	*03:03	282	32.79	361	14.86	$<10^{-26}$	2.80	(2.34–3.35)
	*04:01	222	25.81	317	13.03	$<10^{-15}$	2.32	(1.91–2.82)
	*03:01	31	3.60	282	11.61	$<10^{-11}$	0.29	(0.20–0.42)
	*06:01	21	2.44	440	18.11	$<10^{-36}$	0.11	(0.07–0.18)
	*06:02	0	0.00	151	6.22	$<10^{-19}$	0.00	
	*06:04	37	4.30	167	6.88	NS		
	Others	100	11.63	486	19.98			
DPB1	*02:01	244	28.37	273	21.02	$<10^{-2}$	1.49	(1.22–1.82)
	*03:01	59	6.86	48	3.68	<0.05	1.92	(1.30–2.84)
	*04:02	53	6.16	135	10.40	$<10^{-2}$	0.57	(0.41–0.79)
	*09:01	21	2.44	118	9.12	$<10^{-8}$	0.25	(0.16–0.40)
	*04:01	42	4.88	49	3.80	NS		
	Others	433	50.35	647	26.60			

CI, confidence interval; n, total number of alleles; Pc, corrected p values; OR, odds ratio; NS, not significant.
The total number of alleles in the control data for DRB1 and DQB1 was 2432, while the total number of alleles in the control data for DPB1 was 1298 (21).
Others for DRB1: *01:01, *03:01, *04:01, *04:03, *04:04, *04:07, *04:10, *07:01, *10:01, *11:01, *11:05, *11:06, *12:01, *12:02, *14:01, *14:03, *14:06, *16:02.
Others for DQB1: *02:01, *04:02, *05:01, *05:02, *06:09.
Others for DPB1: *01:01, *02:02, *05:01, *06:01, *13:01, *14:01, *17:01, *19:01, *25:01, *26:01, *29:01, *38:01, *41:01, *48:01.
Corrected p values (Pc), or the p values multiplied by the number of comparisons at each locus, are shown. A Pc value < 0.05 was considered significant.

DRB1*15:01-DQBI*06:02 ($Pc < 10^{-31}$; OR, 0.0), DRB1*15:02-DQBI*06:01 ($Pc < 10^{-14}$; OR, 0.11), and DRB1*08:03-DQBI*06:01 ($Pc < 10^{-6}$; OR, 0.18) (Table 2).

In the transmission disequilibrium test (TDT), the susceptible alleles associated with Type 1A diabetes in Japanese children were DRBI*04:05 ($Pc < 10^{-5}$; OR, 2.83), DRBI*09:01 ($Pc < 10^{-5}$; OR, 2.58), DRBI*08:02 ($Pc < 10^{-3}$; OR, 5.33), DQBI*04:01 ($Pc < 10^{-5}$; OR, 2.76), DQBI*03:03 ($Pc < 10^{-5}$; OR, 2.69), and DQBI*03:02 ($Pc < 10^{-3}$; OR, 2.88) (Table 3). DPB1*02:01 and DPB1*03:01 were not significant when examined using the TDT. The protective alleles were DRBI*15:02 ($Pc < 10^{-6}$; OR, 0.08), DRBI*15:01 ($Pc < 10^{-5}$; OR, 0.00), DRBI*08:03 ($Pc < 0.05$; OR, 0.26), DQBI*06:01 ($Pc < 10^{-9}$; OR, 0.13), DQBI*06:02 ($Pc < 10^{-5}$; OR, 0.00), DQBI*03:01 ($Pc < 10^{-4}$; OR, 0.18), and DPBI*09:01 ($Pc < 10^{-4}$; OR, 0.20); DRB1*04:06 and DPB1*04:02 were not significant when examined using the TDT (Table 3).

Association of HLA-A, C, and B with Type 1A diabetes

In the case-control study, the susceptible alleles associated with Type 1A diabetes in Japanese children were A*24:02 ($Pc < 10^{-2}$; OR, 1.44), C*01:02 ($Pc < 10^{-2}$; OR, 1.56), C*08:01 ($Pc < 0.05$; OR, 1.60), B*07:02 ($Pc < 10^{-3}$; OR, 2.39), B*40:06 ($Pc < 10^{-3}$; OR, 2.21), and B*54:01 ($Pc < 10^{-10}$; OR, 2.82). The protective alleles were A*26:01 ($Pc < 10^{-4}$; OR, 0.43), A*33:03 ($Pc < 10^{-2}$; OR, 0.47), A*11:01 ($Pc < 0.05$; OR, 0.60), C*12:02 ($Pc < 10^{-8}$; OR, 0.28), C*14:03 ($Pc < 10^{-3}$; OR, 0.41), C*15:02 ($Pc < 10^{-3}$; OR, 0.28), B*15:01 ($Pc < 10^{-6}$; OR, 0.30), B*52:01 ($Pc < 10^{-9}$; OR, 0.26), and B*44:03 ($Pc < 0.05$; OR, 0.47) (Table 4).

In the TDT, the susceptible alleles associated with Type 1A diabetes in Japanese children were C*01:02 ($Pc < 10^{-2}$; OR, 1.92), C*08:01 ($Pc < 0.05$; OR, 2.15), and B*54:01 ($Pc < 10^{-5}$; OR, 4.13) (Table 3). The protective alleles were A*33:03 ($Pc < 10^{-2}$; OR, 0.32),

Table 2. Haplotype frequencies of HLA-DRB1-DQB1 among Japanese children with Type 1A diabetes

HLA haplotype	Type 1A		Control			Type 1A vs. Control		
DRB1-DQB1	n = 860	%	n = 1032	%	p	Pc	OR	(95% CI)
*09:01-*03:03	275	31.98	138	13.37	2.19E-22	$<10^{-20}$	3.05	(2.42–3.83)
*04:05-*04:01	222	25.81	134	12.98	1.43E-12	$<10^{-10}$	2.33	(1.84–2.96)
*08:02-*03:02	83	9.65	20	1.94	1.01E-13	$<10^{-11}$	5.41	(3.29–8.89)
*04:05-*03:02	35	4.07	0	0.00	7.05E-13	$<10^{-11}$		
*08:03-*06:01	10	1.16	62	6.01	9.35E-09	$<10^{-6}$	0.18	(0.09–0.36)
*15:02-*06:01	9	1.05	92	8.91	4.24E-16	$<10^{-14}$	0.11	(0.05–0.22)
*15:01-*06:02	0	0.00	118	11.43	3.75E-33	$<10^{-31}$	0.00	
*04:07-*03:02	14	1.63	4	0.39	7.45E-03	NS		
*01:01-*05:01	25	2.91	40	3.88	NS			
*13:02-*06:04	37	4.30	56	5.43	NS			
*15:01-*03:01	4	0.47	2	0.19	NS			
Others	146	16.98	366	35.47				

CI, confidence interval; n, total number of alleles; OR, odds ratio.
The control data were obtained from Ref. (22).

C*12:02 ($Pc < 10^{-5}$; OR, 0.18), C*14:03 ($Pc < 0.05$; OR, 0.33), B*15:01 ($Pc < 0.05$; OR, 0.34), and B*52:01 ($Pc < 10^{-5}$; OR, 0.17) (Table 3).

Linkage disequilibrium (LD) between DRB1-DQB1 haplotypes and DPB1, A, C, or B alleles

DPB1*02:01 and *03:01 were assessed as susceptible alleles in the case-control study but were not specifically associated with any susceptible DRB1-DQB1 haplotype. The RD values for DPB1*02:01 to DRB1*04:05-DQB1*04:01, DRB1*09:01-DQB1*03:03, and DRB1*08:02-DQB1*03:02 were 0.246, 0.312, and 0.112, respectively. The RD values for DPB1*03:01 to DRB*04:05-DQB1*04:01, DRB1*09:01-DQB1*03:03, and DRB1*08:02-DQB1*03:02 were 0.175, 0.081, and 0.148, respectively. A*24:02 was assessed as a susceptible allele in the case-control study but was not specifically associated with any susceptible DRB1-DQB1 haplotype. The RD values for A*24:02 to DRB1*04:05-DQB1*04:01, DRB1*09:01-DQB1*03:03, and DRB1*08:02-DQB1*03:02 were 0.405, 0.310, and 0.286, respectively. However, the susceptible alleles C*01:02 and B*54:01 appeared to be associated with the DRB1*04:05-DQB1*04:01 haplotype (RD, 0.697). Meanwhile, the susceptible C*08:01 and B*40:06 alleles appeared to be associated with the DRB1*09:01-DQB1*03:03 haplotype (RD, 0.597).

DPB1*09:01, C*12:02, and B*52:01 were assessed as protective alleles in both the case-control study and the TDT and appeared to be associated with the protective DRB1*15:02-DQB1*06:01 haplotype. The RD for the C*12:02-B*52:01-DRB1*15:02-DQB1*06:01-DPB1*09:01haplotype was 0.861 among the Japanese children with Type 1A diabetes in this study. The protective alleles A*33:03, C*14:03, and B*44:03 were associated with a high LD (RD, 0.842).

Transmission of susceptible and protective alleles from maternal and paternal parents

In the TDT, the transmission of DRB1*08:02 from the father occurred more frequently than from the mother, but the difference was not significant. The transmission of DRB1*09:01 from the mother occurred more frequently than from the father, but again the difference was not significant. The DRB1, DQB1, and DPB1 alleles were not transmitted preferentially from the mother or father to the children with Type 1A diabetes (Table 3), and the same was true for the A, C, and B alleles (Table 3).

Comparison of combinations of susceptible haplotypes and protective alleles between children with Type 1A diabetes and their parents

When genetic combinations of HLA-DRB1-DQB1 haplotypes were compared between children with Type 1A diabetes and their parents (149 parent–child trios), 54.4% of the children with Type 1A diabetes and 21.3% of their parents had two susceptible haplotypes. The frequencies of DR9/9 (homozygotes for DRB1*09:01-DQB1*03:03) ($Pc < 10^{-2}$; OR, 3.77) in group I (homozygotes for two susceptible haplotypes) and DR4/8 (heterozygotes for DRB1*04:05-DQB1*04:01 and DRB1*08:02-DQB1*03:02) ($Pc < 10^{-2}$; OR, 4.38) in group II (heterozygotes for two susceptible haplotypes) were significantly higher among the children with Type IA diabetes. The frequencies of group IV (one susceptible haplotype and a protective allele) ($Pc < 10^{-10}$; OR, 0.16) and group VI (no susceptible haplotypes and a

Table 3. Transmission disequilibrium test (TDT) for HLA-DRB1, DQB, DPB1, A, C, and B alleles in 149 parent–child trios

		Transmitted			Non-transmitted			TDT				Transmission from Maternal vs. Paternal
			Parent of origin			Parent of origin						
HLA		Combined	Maternal	Paternal	Combined	Maternal	Paternal	P	Pc	OR	(95% CI)	p
DRB1	*04:05	85	41	44	30	12	18	2.92E-07	$<10^{-5}$	2.83	(1.87–4.30)	NS
	*08:02	32	13	19	6	5	1	2.47E-05	$<10^{-3}$	5.33	(2.23–12.76)	NS
	*09:01	85	47	38	33	13	20	1.69E-06	$<10^{-5}$	2.58	(1.72–3.85)	NS
	*08:03	6	1	5	23	13	10	1.59E-03	<0.05	0.26	(0.11–0.64)	NS
	*15:01	0	0	0	26	15	11	3.41E-07	$<10^{-5}$	0.00		NS
	*15:02	3	1	2	39	21	18	2.78E-08	$<10^{-6}$	0.08	(0.02–0.25)	NS
	*04:06	2	2	0	10	4	6	2.09E-02	NS			NS
DQB1	*04:01	80	38	42	29	12	17	1.03E-06	$<10^{-5}$	2.76	(1.80–4.22)	NS
	*03:02	49	28	21	17	9	8	8.18E-05	$<10^{-3}$	2.88	(1.66–5.00)	NS
	*03:03	86	46	40	32	15	17	6.66E-07	$<10^{-5}$	2.69	(1.79–4.03)	NS
	*03:01	7	4	3	40	17	23	1.48E-06	$<10^{-4}$	0.18	(0.08–0.39)	NS
	*06:01	8	2	6	62	34	28	1.09E-10	$<10^{-9}$	0.13	(0.06–0.27)	NS
	*06:02	0	0	0	25	15	10	5.73E-07	$<10^{-5}$	0.00		NS
DPB1	*02:01	63	30	33	43	17	26	NS				NS
	*03:01	23	12	11	14	8	6	NS				NS
	*09:01	7	3	4	35	18	17	1.56E-05	$<10^{-4}$	0.20	(0.09–0.45)	NS
	*04:02	21	10	11	24	14	10	NS				NS
A	*24:02	100	48	52	70	36	34	2.14E-02	NS			NS
	*33:03	9	4	5	28	17	11	1.79E-03	$<10^{-2}$	0.32	(0.15–0.68)	NS
	*11:01	19	11	8	26	15	11	NS				NS
	*26:01	14	9	5	20	10	10	NS				NS
C	*01:02	69	30	39	36	18	18	1.28E-03	$<10^{-2}$	1.92	(1.28–2.87)	NS
	*08:01	43	22	21	20	11	9	3.76E-03	<0.05	2.15	(1.26–3.65)	NS
	*12:02	7	4	3	40	21	19	1.48E-06	$<10^{-5}$	0.18	(0.08–0.39)	NS
	*14:03	8	4	4	24	14	10	4.68E-03	<0.05	0.33	(0.15–0.74)	NS
	*15:02	7	4	3	13	8	5	NS				NS
B	*54:01	62	27	35	15	7	8	8.50E-08	$<10^{-5}$	4.13	(2.35–7.26)	NS
	*40:06	28	14	14	12	3	9	1.14E-02	NS			NS
	*07:02	17	7	10	12	5	7	NS				NS
	*15:01	10	6	4	29	14	15	2.35E-03	<0.05	0.34	(0.17–0.71)	NS
	*52:01	7	4	3	42	23	19	5.73E-07	$<10^{-5}$	0.17	(0.07–0.37)	NS
	*44:03	9	5	4	24	14	10	9.02E-03	NS			NS

CI, confidence interval; OR, odds ratio; TDT, transmission disequilibrium test.

Table 4. HLA-A, C, and B allele frequencies among Japanese children with Type 1A diabetes

		Type 1A		Control		Type 1A vs. Control		
HLA		n = 860	%	n = 1046	%	Pc	OR	(95% CI)
A	*24:02	390	45.35	382	36.52	$<10^{-2}$	1.44	(1.20–1.73)
	*26:01	45	5.23	118	11.28	$<10^{-4}$	0.43	(0.30–0.62)
	*33:03	33	3.84	82	7.84	$<10^{-2}$	0.47	(0.31–0.71)
	*11:01	58	6.74	112	10.71	<0.05	0.60	(0.43–0.84)
	Others	334	38.84	352	33.65			
C	*01:02	204	23.72	174	16.63	$<10^{-2}$	1.56	(1.24–1.95)
	*08:01	127	14.77	102	9.75	<0.05	1.60	(1.21–2.12)
	*12:02	29	3.37	116	11.09	$<10^{-8}$	0.28	(0.18–0.42)
	*14:03	26	3.02	74	7.07	$<10^{-3}$	0.41	(0.26–0.65)
	*15:02	11	1.28	46	4.40	$<10^{-3}$	0.28	(0.14–0.55)
	Others	450	52.33	534	51.05			
B	*07:02	71	8.26	38	3.63	$<10^{-3}$	2.39	(1.59–3.58)
	*40:06	86	10.00	50	4.78	$<10^{-3}$	2.21	(1.54–3.18)
	*54:01	152	17.67	74	7.07	$<10^{-10}$	2.82	(2.10–3.78)
	*15:01	26	3.02	98	9.37	$<10^{-6}$	0.30	(0.19–0.47)
	*52:01	27	3.14	114	10.9	$<10^{-9}$	0.26	(0.17–0.41)
	*44:03	28	3.26	70	6.69	<0.05	0.47	(0.30–0.73)
	Others	470	54.65	602	57.55			

CI, confidence interval; n, total number of alleles; OR, odds ratio.
The control data was obtained from Ref. (22).
Others for A: *01:01, *02:01. *02:06, *02:07, *02:10, *11:02, *24:02, *24:08, *26:02, *26:03, *26:05, *31:01, *32:01.
Others for C: *03:02, *03:03, *03:04, *04:01, *05:01, *06:02, *07:02, *07:04, *08:03, *14:02.
Others for B: *08:01, *13:01, *13:02, *15:02, *15:07, *15:11, *15:18, *27:04, *35:01, *37:01, *38:01, *39:01, *39:02, *39:04, *40:01, *40:02, *40:03, *46:01, *48:01, *51:01, *54:12, *55:02, *55:04, *56:01, *58:01, *59:01, *67:01.

protective allele) (Pc < 0.05; OR, 0.20) were significantly lower among the children with Type 1A diabetes than among their parents (Table 5). Of note, the frequency of group III (one susceptible haplotype and no protective allele) was similar between the children with Type 1A diabetes and their parents (Table 5).

GADAb and/or IA-2Ab were positive in 21 (7.1%) of the 296 parents: one in group I, five in group II, six in group III, four in group IV, three in group V, and two in group VI. Three parents (1.0%) had type 1 diabetes mellitus: two in group II and one in group III.

Comparison between children with Type 1A diabetes and their siblings

When the frequencies of the HLA-DRB1, DQB1, and DPB1 alleles were compared between 66 children with Type 1A diabetes and their 83 healthy siblings, the prevalences of all the alleles except for DQB1*06:01 were not significantly different. The frequency of the DQB1*06:01 protective allele was lower (Pc < 10^{-2}; OR, 0.13) among the patients than among their siblings.

When genetic combinations of HLA-DRB1-DQB1 haplotypes were compared between children with Type 1A diabetes and their siblings, the frequency of group VI (no susceptible haplotypes and a protective allele) was lower (Pc < 10^{-2}; OR, 0.09) among the children with Type 1A diabetes (3.03%) than among the siblings (25.3%) (Table 6). Of note, 44.6% of the siblings had protective alleles (groups IV + VI), compared with 10.6% of the children with Type 1A diabetes.

GADAb and/or IA-2Ab were positive in 7 (8.4%) of the 83 siblings: three in group II, three in group III, and one in group V. Groups II, III, and V can be characterized as having no protective alleles.

Onset age and HLA genotype

The DRB1 allele frequencies in four age groups, determined according to the patient's age at the time of Type 1A diabetes onset (0–1, 2–5, 6–9, and 10–16 years), are shown in Fig. 1. The frequency of DRB1*09:01 was higher (Pc < 0.01) in the 2–5-year onset group than in the other age groups, while the frequency of DRB1*08:02 tended to be higher in the 6–16-year onset group, although the difference was not significant (Fig. 1). The distribution of the DRB1*04:05 allele was not different among the four age groups. The distributions of other alleles, including DPB1*02:01, DPB1*03:01, A*24:02, C*01:02, C*08:01, and B*54:01, were not different among the four age groups (data not shown).

Discussion

This study is the first nationwide multicenter collaborative study examining genetic factors associated with

Table 5. Genetic combinations of HLA-DRB1-DQB1 haplotypes in Japanese children with Type 1A diabetes and their parents

Genetic combination of	Type 1A all		Type 1A in trio		Parents in trio		Type 1A in trio vs. Parents			
HLA-DRB1-DQB1 haplotype	n = 430	%	n = 149	%	n = 296	%	p	Pc	OR	(95% CI)
I. Two susceptible haplotypes in homozygote	82	19.07	37	24.83	21	7.09	4.33E-07	$<10^{-5}$	4.33	(2.43–7.72)
DR4/4 (*04:05-*04:01)	8	1.86	7	4.70	5	1.69	NS			
DR4/4 (*04:05-*03:02)	10	2.33	6	4.03	2	0.68	1.92E-02	NS		
DR9/9 (*09:01-*03:03)	58	13.49	22	14.77	13	4.39	2.68E-04	$<10^{-2}$	3.77	(1.84–7.72)
DR8/8 (*08:02-*03:02)	6	1.40	2	1.34	1	0.34	NS			
II. Two susceptible haplotypes in heterozygote	143	33.26	44	29.53	42	14.19	1.96E-04	$<10^{-2}$	2.53	(1.57–4.10)
DR4/9	65	15.12	19	12.75	26	8.78	NS			
DR4/8	61	14.19	18	12.08	9	3.04	4.59E-04	$<10^{-2}$	4.38	(1.92–10.01)
DR9/8	17	3.95	7	4.70	7	2.36	NS			
III. One susceptible haplotype and no protective allele	135	31.40	44	29.53	66	22.30	NS			
DR4/X	62	14.42	23	15.44	23	7.77	1.99E-02	NS		
DR9/X	64	14.88	18	12.08	32	10.81	NS			
DR8/X	9	2.09	3	2.01	10	3.38	NS			
IV. One susceptible haplotype and a protective allele	43	10.00	15	10.07	121	40.88	2.62E-12	$<10^{-10}$	0.16	(0.09–0.29)
V. No susceptible haplotype and no protective allele	13	3.02	6	4.03	19	6.42	NS			
VI. No susceptible haplotype and a protective allele	14	3.26	3	2.01	28	9.46	2.66E-03	<0.05	0.20	(0.06–0.66)

CI, confidence interval; OR, odds ratio.
Susceptible haplotype: *04:05-*04:01, *09:01-*03:03, *08:02-*03:02, *04:05-*03:02.
Protective allele in DRB1: *08:03, *15:01, *15:02, *04:06.
Protective allele in DQB1: *06:01, *06:02, *03:01.
X in DRB1: *01:01, *03:01, *04:01, *04:03,*04:04,*04:07, *04:10, *07:01,*10:01, *11:01, *11:06, *12:01, *12:02, *13:02, *16:02.
X in DQB1: *02:01, *04:02, *05:01, *05:02, *06:04, *06:09.