conducted this study and collected the data. T. S., M. H., T. M. and H. F. conducted this study, carried out data collection and analysed the manuscript. K. T. carried out the analysis. Y. Y. contributed to design, analysis and writing of the manuscript.

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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.
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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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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 DRBI*09:01 ($Pc < 10^{-29}$; OR, 3.00), DRBI*04:05 ($Pc < 10^{-20}$; OR, 2.60), DRBI*08:02 ($Pc < 10^{-12}$; OR, 3.11), DQBI*03:03 ($Pc < 10^{-26}$; OR, 2.80), DQBI*04:01 ($Pc < 10^{-16}$; OR, 2.32), DQBI*03:02 ($Pc < 10^{-12}$; OR, 2.34), DPBI*02:01 ($Pc < 10^{-2}$; OR, 1.49), and DPBI*03:01 ($Pc < 0.05$; OR, 1.92). The protective alleles were DRBI*15:02 ($Pc < 10^{-21}$; OR, 0.09), DRBI*15:01 ($Pc < 10^{-16}$; OR, 0.06), DRBI*08:03 ($Pc < 10^{-14}$; OR, 0.14), DRBI*04:06 ($Pc < 10^{-3}$; OR, 0.23), DQBI*06:01 ($Pc < 10^{-36}$; OR, 0.11), DQBI*06:02 ($Pc < 10^{-19}$; OR, 0.00), DQBI*03:01 ($Pc < 10^{-11}$; OR, 0.29), DPBI*09:01 ($Pc < 10^{-8}$; OR, 0.25), and DPBI*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-DQBI*03:03 ($Pc < 10^{-20}$; OR, 3.05), DRB1*04:05-DQBI*04:01 ($Pc < 10^{-10}$; OR, 2.33), DRB1*08:02-DQBI*03:02 ($Pc < 10^{-11}$; OR, 5.41), and DRB1*04:05-DQBI*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

HLA		Transmitted			Non-transmitted			TDT				Transmission from Maternal vs. Paternal
			Parent of origin			Parent of origin						
		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.

Table 6. Genetic combinations of HLA-DRB1-DQB1 haplotypes in Japanese children with Type 1A diabetes and their siblings

Genetic combination of	Type 1A		Siblings		Type 1A vs. Siblings			
HLA-DRB1-DQB1 haplotype	n = 66	%	n = 83	%	p	Pc	OR	(95% CI)
I. Two susceptible haplotypes in homozygote	16	24.24	11	13.25	NS			
DR4/4 (*04:05-*04:01)	4	6.06	3	3.61	NS			
DR4/4 (*04:05-*03:02)	3	4.55	2	2.41	NS			
DR9/9 (*09:01-*03:03)	9	13.64	6	7.23	NS			
DR8/8 (*08:02-*03:02)	0	0.00	0	0.00	NS			
II. Two susceptible haplotypes in heterozygote	19	28.79	17	20.48	NS			
DR4/9	9	13.64	13	15.66	NS			
DR4/8	8	12.12	2	2.41	2.31E-02	NS		
DR9/8	2	3.03	2	2.41	NS			
III. One susceptible haplotype and no protective allele	20	30.30	15	18.07	NS			
DR4/X	9	13.64	5	6.02	NS			
DR9/X	10	15.15	7	8.43	NS			
DR8/X	1	1.52	3	3.61	NS			
IV. One susceptible haplotype and a protective allele	5	7.58	16	19.28	NS			
V. No susceptible haplotype and no protective allele	4	6.06	3	3.61	NS			
VI. No susceptible haplotype and a protective allele	2	3.03	21	25.30	1.50E-04	$<10^{-2}$	0.09	(0.02–0.41)

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.

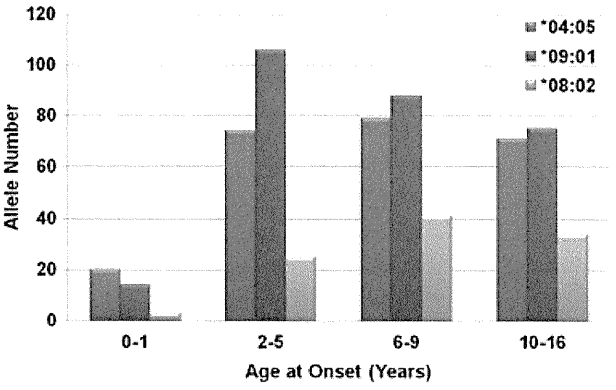

Fig. 1. DRB1 allele frequencies in four age groups of Japanese children with Type 1A diabetes according to the age at onset. The frequency of DRB1*09:01 was higher (Pc < 0.01) in the 2–5-year onset group, while the frequency of DRB1*08:02 tended to be higher in the 6–16-year onset group, although the difference was not significant. The distribution of the DRB1*04:05 allele frequency was not different among the four age groups.

childhood-onset type 1 diabetes mellitus in Japan. In the study, a TDT was performed for the first time in a population of Japanese children with Type 1A diabetes; the results confirmed the identities of susceptible and protective DRB1, DQB1, DPB1, A, C, and B, alleles.

We confirmed previously reported HLA-DRB1-DQB1 susceptible and protective haplotypes and obtained new findings regarding the DPB1 allele. DPB1*02:01 and DPB1*03:01 were identified as susceptible alleles among Japanese children with Type 1A diabetes (Table 1). DPB1*02:01 is unique to the Japanese population, while DPB1*03:01 is observed in multiple ethnic groups (4, 5, 16–19). This finding is noteworthy because neither the susceptible DRB1 allele nor the DQB1 allele is common to both Japanese and Caucasian populations. Moreover, the DPB1*02:01 and DPB1*03:01 alleles were not specifically associated with any susceptible DRB1-DQB1 haplotype. DPB1*04:02 was identified as a protective allele among Japanese children with Type 1A diabetes, similar to cases in multiple ethnic groups (4, 5, 16–19). Moreover, the DPB1*04:02 allele was not associated with any protective DRB1-DQB1 haplotypes. Of note, however, the association of DPB1*02:01, DPB1*03:01, and DPB1*04:02 with Type 1A diabetes was relatively weak in Japanese children, as the association was significant in the case-control study but not in the TDT. However, DPB1*09:01 was identified as a protective allele in both the case-control study and the TDT,

and DPB1*09:01 appeared to be associated with the protective DRB1*15:02-DQB1*06:01 haplotype, which is a major protective haplotype in the Japanese population but is rare in Caucasian populations.

The independent effects of HLA-A and B have been demonstrated in Caucasian populations (2, 4, 5). Following adjustment for LD to haplotypes at the DR-DQ region, both susceptible and protective alleles were found at HLA-A (e.g., A*24:02, susceptible allele; A*11:01, protective allele) and HLA-B (e.g., B*39:06, susceptible allele; B*57:01, protective allele) (4, 5). A*24:02 was a susceptible allele independent of the susceptible DRB1-DQB1 haplotypes among Japanese children with Type 1A diabetes. A*11:01 was also a protective allele among Japanese children with Type 1A diabetes. However, the association of A*24:02 and A*11:01 with Type 1A diabetes was relatively weak in the Japanese children, as the association was significant in the case-control study but not in the TDT (Tables 3 and 4). Of note, the B*39:06 and B*57:01 alleles were not observed in this study.

The analysis of LD between DRB1-DQB1 haplotypes and DPB1, A, C, or B alleles demonstrated both susceptible (C*08:01-B*40:06-DRB1*09:01-DQB1*03:03 and C*01:02-B*54:01-DRB1*04:05-DQB1*04:01) as well as protective (C*12:02-B*52:01-DRB1*15:02-DQB1*06:01-DPB1*09:01 and A*33:03-C*14:03-B*44:03) haplotypes among Japanese children with Type 1A diabetes.

In terms of genomic imprinting of the HLA-class II gene, several studies have been reported (26–29). In a Caucasian population, a striking feature of the data was that HLA-DR3/DR4 patients inherit their DR3 allele from their mother and the DR4 allele from their father more often than vice versa. Margaritte-Jeannin et al. (27) proposed that parental imprinting for a specific allelic combination may explain the HLA genotypes observed in the patients and their relatives. Sadauskaite-Kuehne et al. (28) also studied diabetes-associated allelic transmission rates from mothers and fathers to children with diabetes in 125 families in Lithuania, an area with a low incidence of type 1 diabetes. They reported that the DR4-DQB1*03:02-DQA1*03:01 haplotype was transmitted significantly more frequently from both parents, but that the DR3-DQB1*02:01-DQA1*05:01 haplotype was transmitted more frequently from only mothers. In Japan, Sasaki et al. (29) reported that maternal alleles in a susceptible DQA1*03:01-DQB1*03:02 haplotype showed a strong transmission disequilibrium with GADAb-positive type 1 diabetes, while paternal alleles in the same haplotype did not in 28 nuclear families, supporting the hypothesis that an epigenetic mechanism including genomic imprinting at the HLA-DQ region is involved in the pathogenesis and the genetic complexity of Japanese type 1 diabetes. However, none of the DRB1, DQB1, DPB1, A, C, or B alleles were preferentially transmitted from the mother or the father to the children with Type 1A diabetes in this study (Table 3). Our study suggests that the genomic imprinting of HLA-class II and class I genes is not involved in the pathogenesis of Type 1A diabetes in Japanese patients.

The frequency of subjects with two susceptible DRB1-DQB1 haplotypes was significantly higher among the children with Type 1A diabetes than among their parents. Of note, the frequencies of homozygosity for DRB1*09:01-DQB1*03:03 and of heterozygosity for DRB1*04:05-DQB1*04:01 and DRB1*08:02-DQB1*03:02 were significantly higher among children with Type IA diabetes, while the frequency of subjects with one susceptible haplotype and without a protective allele (group III) was not different between children with Type 1A diabetes and their parents. The frequencies of subjects with one susceptible haplotype and a protective allele (group IV) and with no susceptible haplotype and a protective allele (group VI) were lower among the children with Type 1A diabetes than among their parents (Table 5). These results suggest a dose effect of susceptible DRB1-DQB1 haplotypes and the effect of protective alleles.

The siblings of children with Type 1A diabetes may also represent a high-risk group for type 1 diabetes in the Japanese population, as the high prevalence (about 4%) of diabetes among Japanese siblings is comparable with that among Caucasian siblings (about 6%) (7, 8). The prevalences of the susceptible DRB1 and DQB1 alleles were similar between the children with Type 1A diabetes and their siblings. However, the prevalence of the protective DQB1*06:01 allele was higher among non-diabetic siblings. The frequency of group IV (no susceptible haplotype and a protective allele) was higher among the siblings than among the children with Type 1A diabetes. These results suggest the role of the protective allele among the siblings.

Only the allele frequency of DRB1*09:01 was significantly different among four age groups of Japanese children with Type IA diabetes determined according to the age at the time of onset (0–1, 2–5, 6–9, and 10–16 years). DRB1*09:01 may be strongly associated with an early onset in preschool children, whereas DRB1*08:02 may be weakly associated with a later onset in school-age children. Murao et al. (15) focused on the differences in the contributions of HLA-DR and -DQ haplotypes to the susceptibility to Type 1 diabetes during adulthood (later than 20 years of age) and childhood (1.0–18 years of age) in Japanese patients. They reported that the DRB1*09:01-DQB1*03:03 (DR9) frequency/DRB1*04:05-DQB1*04:01 (DR4) frequency increased with an increasing age of onset, and that another susceptible haplotype, DRB1*08:02-DQB1*03:02 (DR8), was involved only in the childhood-onset group. They did not mention any

difference among childhood-onset type 1 diabetes, and our results complement the data reported by Murao et al. The present results are also compatible with and complementary to our previous report, in which the frequency of the DR9 genotype was found to be significantly higher among a younger age group (0–10 years) than among an older age group (11–16 years) at the time of onset, and the frequency of DR4-DQ4 was higher in the older age group (11–16 years) (13).

Kawabata et al. (30) reported the age-related association of the MHC class I chain-related gene A and a marker in the class I C region with Japanese type 1 diabetes. However, this study did not show an association of susceptible class I A*24:02, C*01:02, C*08:01, or B*54:01 alleles with age at the time of onset in children with Type 1A diabetes (data not shown).

The amino acid residue at position 57 of the DQβ chain has been shown to play a key role in genetic susceptibility to type 1 diabetes. The lack of aspartic acid at this position at both DQ alleles is strongly associated with type 1 diabetes in Caucasian populations (31, 32). However, this Asp57 hypothesis is not tenable for Japanese type 1 diabetic patients (33). The influence of the HLA-DR and HLA-DQ molecules on the risk of type 1 diabetes is probably related to their central role in antigen presentation and the activation of a helper T cell-mediated immune response (2, 32). The HLA-class II and class I pocket structure is critical to the etiology of autoimmunity, as different pocket variants may have different affinities to the antigenic peptides of specific proteins from pancreatic β cells, including insulin and GAD; therefore, certain variants are more likely to present autoantigenic peptides to T cells than others (32, 34). In a future study, an analysis of how variations in amino acids, especially those found within the peptide-binding domains, are correlated with changes in disease risk would be valuable, providing a possible link between genetic association studies and the causal mechanism(s) of Type 1A diabetes.

In conclusion, this study demonstrated the characteristic association, which was mostly different but partly the same as that in Caucasian populations, of HLA-DRB1, DQB1, DPB1, and A, C, B, 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 in the present population. A comparison of children with Type 1A diabetes and their parents and siblings suggested a dose effect of susceptible DRB1-DQB1 haplotypes and the effect of protective alleles on the immunological pathogenesis of Type 1A diabetes. These results may provide fundamental data for further genetic studies examining other immune-related and insulin resistance or beta cell function-related genes in Japanese patients with type 1 diabetes.

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Conflict of interest

No potential conflicts of interest relevant to this article were present.

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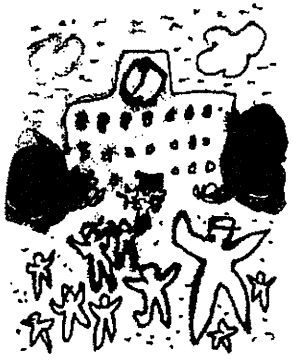
毎月ひとつの病気を取り上げ、学校・保護者・医療機関の連携や子どものQOL向上への要点などについて専門医師が解説します。

糖尿病の子どもと学校教育

雨宮 伸



子どもの糖尿病とは

(1)成因

糖尿病は、成因によって大きくは「1型」「2型」「その他の特定の機序、疾患によるもの」に分類されます。子どもでもこの分類があてはまりますが、従来、子どもの糖尿病というと1型と考えられていました。1型糖尿病は、発症年齢が幼児期からのことも珍しくなく、生活習慣やいわゆる遺伝疾患とは直接には関係ありません。膵臓のβ細胞を自分で破壊してしまう自己免疫機序が主な原因です（機序：しくみ、操作＝編集部注）。膵臓からのインスリン分泌が枯渇していく病態が基本となりますので、インスリンの注射によってこれを補います。

注射は、各食事やおやつからの栄養を体に取り込むための食事時の「追加インスリン」といわれるものと、食事の間や夜間に脳や神経・血球などへ一定のブドウ糖（血糖）を供給するため肝臓からのブドウ糖の産生にブレーキ役となる「持続インスリン」が基本となります。つまり、一日数回の注射により血糖コントロールは維持され、このような強化インスリン療法の進歩により合併症の進展抑制は急速に改善されてきています。

2型糖尿病は従来、成人糖尿病と思われていましたが、日本では三十年前ほどから思春期以降での発症増加が認められ、肥満との関連が指摘されています。その発症の要因は、インスリン抵抗性（インスリンの効果が出にくくなること）の増大と、それを代償する（補う）ためのインスリン分泌の増加が破綻をきたすことにあります。

(2)発症リスク

人種的にも発症のリスクは異なります。欧米白人は1型糖尿病の発症リスクは日本人の二〇～三〇倍と高いのですが、2型糖尿病は近年肥満の増大が社会現象となって小児・思春期でも発症が増加しています。一方、日本人やアジア人はそれほど肥満が強くなくても、思春期での2型糖尿病が見られます。つまり、日本人ではインスリンの分泌能力が人種的に少ないので、小太り程度の肥満でもリスクとなっているのです。思春期糖尿病の二～三割は非肥満であることに注意が必要です。

また、インスリン抵抗性の増大は、肥満ばかりではありません。思春期は成長するための成長ホルモンの分泌が増えますが、このホルモンはインスリン抵抗性を生理的に作ることになるので、2型糖尿病はほとんど思春期以降に発症します。日本人では思春期の糖尿病は1型より2型糖尿病のほうが発症率は高いといわれています。

思春期2型糖尿病は、その両親および祖父母の半数以上に糖尿病の家族歴があります。しかし、特定の遺伝子が成因であることは稀で、生活習慣を含めた環境因子も発症リスクに関連する、多因子疾患です。最近は、出生時体重が二五〇〇グラム未満の子どもが年々増加しています。この低出生体重児は将来、2型糖尿病も含めた生活習慣病

＊「教育と医学」2010年6月号「特集2・慢性疾患をもつ子どもと学校教育」で現状や問題点を解説しています。併せてご活用ください（http://www.keio-up.co.jp/np/inner/30684/）。

になりやすいことも判ってきており、子宮内の胎児環境と出生後の環境の相違が、生活習慣病に関連する遺伝子に後天的に変化を与えているとも考えられています。

このように2型糖尿病の発症には、肥満をはじめとするインスリン抵抗性の増大がありますので、肥満の解消にむけた食事・運動療法が指導の基本になります。2型糖尿病となると、心・血管病変進展のリスクが高まりますので、肥満がなくても食事・運動療法は重要です。

一方、日本人は肥満であっても基本的にインスリン分泌の代償機能は少ないので、血糖コントロールに薬物療法が必要となることも多いのです。現実に思春期発症2型糖尿病の予後は1型糖尿病に比べ良くないことが知られ、網膜症、腎症など糖尿病性慢性合併症が三十歳などの若年成人で既に顕在化し、社会・経済生活に支障をきたしてしまっている場合も少なくありません。

つまり、自覚症状のないことの多い2型糖尿病では、徒に食事・運動療法に拘泥することなく、血糖コントロールが不十分であれば積極的な薬物療法の導入も必要となります。

学校生活における問題点とその対応

(1)1型糖尿病の場合

1型糖尿病においては、インスリン注射が最も子どもたちを悩ます問題です。ひんぱんな回数によるインスリン注射による強化療法が将来の合併症予防に明らかに効果があることが判っていますので、学校での受け入れ体制の不備によるインスリン注射の回避は望ましくありません。インスリン注射量の設定は、最近「カーボカウント」により決めることが多くなっています。食事内容における糖質（カーボ）量に応じたインスリン量の比によって、各食事での超速効型インスリンの注射量が決まります。したがって、給食の内容はカロリーのみならず、糖質量も予め家庭に知らされていることが必要になります。

年少児ではインスリン注射が自分でできないこともありますし、注射量の決定も難しいこともあります。そこで、昼間に注射をしない方法を選ぶことも、家族や本人の希望なら仕方ありません。インスリン注射回数や製剤の選択の権利は、本人と保護者にあります。裏返せば、将来を見すえた治療法の的確な選択へ導いてあげられるかは、医療側の科学的根拠に基づいた情報の提供と、学校での積極的対応にかかっています。

最近は超速効型インスリンのみを使って、ポンプを携帯してもらう「持続皮下インスリン注入療法（CSII）」が年少者にも導入されることがあります。食事・間食での頻回注射はかなり負担ですので、三日に一回程度皮下にカニューレ（管）を刺し入れます。食事追加インスリンも基礎インスリンもすべて、ポンプを操作してこのカニューレから投与されますので、一日数回の自己注射の必要はなくなります。いろいろな投与法をプログラムすることができますので、昼食時インスリンへの対応を家庭で設定しておくこともあります。当然、ポンプの携帯による不便も予測されますが、子どのたちの対応と受け入れは頻回注射（一日に何度か注射をする）に優るものがあります。

以上のように、学校でのインスリン注射は必須と考えるべきであり、注射をする場所の選択がしばしば問題となります。本人の了解が得られれば、教室内やどこでも注射に問題はありません。ある程度の遮蔽は必要かもしれませんが、トイレに入って注射をする子どもが多いのは改善すべき課題の一つです。また、保健室にわざわざ出向くことも少なくありませんが、その必要性は少ないと思われます。インスリン注射さえできれば、1型糖尿病の子どもに何ら学校生活への制限はありません。友達に理解を得られる状況を学校が提供できるか否かは、本人のQOL向上の基本となると考えられます。

(2)2型糖尿病の場合

2型糖尿病への学校における問題は、家庭・社会における背景も考慮する必要があり、複雑です。特に肥満を伴う子どもたちは、糖尿病の診断の前に、身体・精神的な問題を抱えていることも少なくありません。つまり、生活習慣病となる本人の成育歴の上に糖尿病が診断され、また多くの場合、明確な自覚症状を欠いていることが多いの

です。特に、学校健診における尿糖スクリーニングにおいては、糖尿病診断までとその後の療養の手引きに対する事後処置が大変曖昧なままとなっています。糖尿病のレッテルを貼られ、さらに肥満であるのは本人の生活習慣、家族の教育が悪いとしかとらえられていない場合が少なくありません。不登校・引きこもり、いじめ、片親、低収入家庭、糖尿病の家族歴など、糖尿病診断前にすでにこういった問題を抱えていることは稀でありません。

また、2型糖尿病は自覚症状が少ないため、医療機関への継続的受診が中断してしまうことも少なくありません。特に肥満2型糖尿病の診断当初は、食事および運動の療養指導のみでも血糖コントロールは一見正常化することも少なくありません。しかし、年余にわたって安定することは少なく、寛解（完全治癒ではないが、症状が軽減または消失すること）したと錯覚したり、その後の治療にむしろ難渋することもあります。継続的な受診と、解決すべき問題点と目標を明確にしてあげる必要があります。継続受診には、本人および家族への治療意欲への励ましが必須です。

学校と医療機関の連携

(1)1型糖尿病の場合

1型糖尿病における学校との連携で最も気になるのが、低血糖への対処です。低血糖となると本人自身で自分がどんな状況か判断できなくなったり、時には痙攣または意識喪失に至ることもあります。しかし、重症に見えてもほとんどの場合、回復させることができます。つまり、ブドウ糖、ペットシュガーなどの携帯または保管場所を決めておけば、無理やり口に押し込めば自然に飲みこみます。また、交感神経が亢進（高ぶり進む）し、糖新生（血中の糖が少なくなったとき、筋肉を分解し肝臓でグリコーゲン以外のものからグルコースを合成しエネルギーを作り出す現象）も回復してきます。ジュースなどは投与しやすいですが、血糖回復には時間がかかります。当然、重症なら近くの学校医やかかりつけ医でブドウ糖の注射やグルカゴン注射をできる体制を話し合っておくこともよいでしょう。

一方、低血糖は多くが自覚できます。体のだるさ、ふるえ、イラつき、冷や汗、動悸、目のかすみ、空腹感、注意力低下、顔面蒼白などです。本人が低血糖と感じたら、自分でブドウ糖、ペットシュガーなどを摂取できる環境を教師や友達に作っておいてもらえることが必要です。登下校、とくに昼の給食がない日やクラブ活動後など低血糖になりやすい場合を考え、友達に低血糖の症状を知っておいてもらうと安心です。

さらに、遠足など運動が長時間になるときは低血糖になりやすいので、予め投与するインスリン量を減らしておく必要があります。また、低血糖を防ぐため糖質を主としたおにぎりやクッキーなどを繰り返し摂取する必要があることも周知しておいてほしいことです。

また、年少者や糖尿病になって間もない時期は低血糖かどうかわからないこともあります。1型糖尿病では血糖を自分で測ることができます。この血糖自己測定を学校でもできるようにしておけば、療養体制はさらに充実するとも考えられます。

(2)2型糖尿病の場合

2型糖尿病については、継続受診と治療の遵守が予後改善の軸となります。社会・学校・家庭での課題をもつことが少なくないので、生活習慣が是正できないことを徒に非難することは、かえって療養継続の意欲をなくさせてしまいます。継続受診ができているか、学校でも食事療法・服薬が遵守できているか、励ますことの環境整備が必須です。また、肥満者では体重管理（日々の体重測定の励行）は生活習慣の改善の重要なカギになっています。一方、2型糖尿病でもインスリン注射が必要なことはあります。この場合、1型糖尿病に準じた連携も必要です。

2型糖尿病の発症リスクのひとつに、母体の痩せと妊娠中の至適体重増加の不良が問題となっています。これは、学校保健教育における課題です。ここ数年の学校保健統計では肥満児の増加抑制または軽度減少が認められてきていますが、肥満のみにとらわれず、小児メタボリックシンドロームをきちんと日本的意義を見据えて再検討すべきと考えています。

保護者との連携

(1) 1型糖尿病の場合

1型糖尿病での発症時の不安は、本人および保護者にとって大変衝撃となります。学校生活を無事過ごせるかの心配をいかに受け止めてあげるかで、その後の療養の充実に大きな違いが出ます。将来を見据えた自己管理の習熟を見守る体制について、本人、保護者、学校および医療関係者間で話し合えれば、現在のインスリン治療は学校生活や社会生活に応じた対応を可能としています。インスリンを注射するから何を食べてはいけない、低血糖が起こるかもしれないから何ができない、ということはありません。インスリンさえ適切に投与できれば、他に何も制限は必要ありません。

年少時に発症した1型糖尿病における問題のひとつに、自立の遅れがあります。インスリン投与量の設定や注射に保護者の介助を必要としていた子どもにとって、昼食への対応や補食についての自立には学校側との連携は不可欠です。いつまでも保育園、幼稚園、学校へ家族が出向くのは、子どもの自立にとっても不自然ですし、友人関係の促進に妨げとなります。

1型糖尿病においては、糖尿病キャンプが全国的に各地で開催されており、年少者への自立支援、自己血糖測定、自己注射、さらに1型糖尿病の仲間作りが企画されています。1型糖尿病の年少者もこのような環境の中にいると、自立はそんなに難しいことではないことが観察されます。学校関係者も過度に防衛的にならず、また家族も過度に保護的または学校への過度の期待を前提としないでも、子どもたちは常に自立の用意があると考えられます。

(2) 2型糖尿病の場合

2型糖尿病ではやはり、本人および家族全体への支援体制を、学校のみならず社会の問題として考える必要があります。小児慢性特定疾患支援事業のなかに糖尿病は含まれますが、2型糖尿病については薬物療法を受けていることが前提になり、支援の体制が不十分になりがちなことは否め

「糖尿病患児の治療・緊急連絡法等の連絡表」　（日本学校保健会の提供による）

糖尿病患児の治療・緊急連絡法等の連絡表

学校名　　年　組　　記載日　平成　年　月　日
医療機関
氏名　　男・女　　医師名　　印
生年月日　昭和・平成　年　月　日　　電話番号

要管理者の現在の治療内容・緊急連絡法

診断名　①1型（インスリン依存型）糖尿病　②2型（インスリン非依存型）糖尿病
現在の治療　1. インスリン注射：　1日　回　　昼食前の学校での注射（有・無）
学校での自己血糖値測定（有・無）
2. 経口血糖降下薬：薬品名（　　）学校での服用（有・無）
3. 食事・運動療法のみ
4. 受診回数　　回／月
緊急連絡先　保護者　氏名　　自宅TEL
勤務先（会社名　　TEL　　）
主治医　氏名　　施設名　　TEL

学校生活一般：基本的には健常児と同じ学校生活が可能である

1. 食事に関する注意
学校給食　①制限なし　②お代わりなし　③その他（　　）
宿泊学習の食事　①制限なし　②お代わりなし　③その他（　　）
補食　①定時に（　時　食品名　　）
②必要なときのみ（どういう時　　）
（食品名　　）
③必要なし
2. 日常の体育活動・運動部活動について
「日本学校保健会　学校生活管理指導表」を参照のこと
3. 学校行事（宿泊学習、修学旅行など）への参加及びその身体活動
「日本学校保健会　学校生活管理指導表」を参照のこと
4. その他の注意事項

低血糖が起こったときの対応*

程度	症状	対応
軽度	空腹感、いらいら、手がふるえる	グルコース錠2個（40kcal＝0.5単位分。入手できなければ、スティックシュガー10g）
中程度	黙り込む、冷汗・蒼白、異常行動	グルコース錠2個（あるいは、スティックシュガー10g） さらに多糖類を40～80kcal（0.5～1単位分）食べる。（ビスケットやクッキーなら2～3枚、食パンなら1/2枚、小さいおにぎり1つなど） 上記補助食を食べた後、保健室で休養させ経過観察する。
高度	意識障害、けいれんなど	保護者・主治医に緊急連絡し、救急車にて主治医または近くの病院に転送する。救急車を待つ間、砂糖などを口内の頬粘膜になすりつける。

＊軽度であっても低血糖が起こったときは、保護者・主事医に連絡することが望ましい。

ません。生活習慣の是正が必要な場合、その家族内では解決できない問題も少なくありません。2型糖尿病については、キャンプのような療養指導の場も少なく、経済的負担からキャンプのような場が企画されても参加は容易ではありません。また、単なる肥満防止キャンペーンでは、現実の2型糖尿病の子どもへの支援としては不十分です。小児科医、内科医、糖尿病・内分泌専門医が今後さらに真剣に取り組むべき時期にきているようです。

子どものQOL向上のため

糖尿病の子どもたちおよび保護者のQOLを同年齢の非糖尿病の子どもと比較した研究がなされています。詳細は報告書を参考にしてください。日本でのその報告の内容から筆者が感じていることを若干述べます。

1型糖尿病の子どもたちのQOLは、子どもも保護者も総じて悪くないとされています。前述してきたように、1型糖尿病の少ない日本では、欧米に比べ社会・学校での理解が乏しいぶん、保護者が懸命に支援体制を連携して構築していると思われます。また、全国各地での糖尿病サマーキャンプも日本糖尿病協会が中心となって行われるようになり、財政的支援もある程度あります。キャンプの運営も医療関係者から保護者主体になりつつあり、キャンプ参加の経験のある青年たちがキャンプの運営に協力している姿が多くなりました。

しかし、欧米のように1型糖尿病の子どもが周囲に多くいる社会でのQOLに比して、日本では決して満足がいくものとしては報告されていません。ここには、血糖コントロールを改善する様々な特徴を持つインスリン製剤の開発やポンプや人工膵臓などの治療技術の向上のみでは解決できない患者および保護者の苦悩が存在し、QOLの改善にはまだ遠いことを意味していると考えます。そして、日本におけるQOLの見かけ上の満足は、やはり子どもたちの自立に対し、我々医療者、社会、学校、保護者、本人に未熟な面が残されている裏返しかもしれないと考えています。

国際的にも、QOLの向上をめざした取り組みが国際糖尿病連合（IDF）と国際小児・思春期糖尿病学会（ISPAD）においてDAWN YOUTH (DAWN: Diabetes Attitudes, Wishes and Needs) として行われており、日本からも参加して意見交換がなされています。また、肥満の増加と小児2型糖尿病の増加は「パンデミック」として注目を浴びている世界的な課題です。共通の認識はありますが、この子どもたちのQOLの低さは、経済格差の広がりとも関連があり、容易に改善の道は拓かれないと思われます。糖尿病と貧困の再生産に陥らないよう、世代を超えてライフサイクルのどの時点でも有効な介入が期待されます。

糖尿病は、1型であれ2型糖尿病であれ、早期の血糖管理がその後十数年にわたっての合併症進展に影響することが判ってきました。このような早期血糖管理の遠隔効果をそれぞれ、「メタボリック　メモリー」および「遺産効果（legacy effect）」といいます。残された人生を糖尿病と付き合っていかねばならない小児・思春期発症糖尿病をもつ子どもたちへの支援は、本人、家族のみならず、学校・社会全体が果たす役割が大きいのです。

[参考図書]
1)日本小児内分泌学会糖尿病委員会編『こどもの1型糖尿病ガイドブック――患児とその家族のために』文光堂、二〇〇七年
2)日本糖尿病学会（編）『小児・思春期糖尿病管理の手びき』南江堂、二〇〇七年
3)雨宮伸「新時代の糖尿病学(3)：小児糖尿病患者・家族への教育」、『日本臨床』66：suppl7, pp497-501, 二〇〇八年

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＊次回は「てんかんの子どもと学校教育」です。

各論

膵β細胞死

太田康晴[1)]／谷澤幸生[2)]

(SUMMARY) 膵β細胞量は，複製，アポトーシス，新生のバランスによって決定されるが，2型糖尿病における膵β細胞量の減少はアポトーシスの増加によるとされている．しかし，実際にはアポトーシスの定量は困難であるため，残存膵β細胞量を解析することで，アポトーシスの定量を代用するというのが現状である．グルコース負荷後のインスリン反応が，空腹時のデータを元に算出するHOMA-βなどに比べて，より鋭敏に膵β細胞量を反映する指標であることが示されている．現時点では，*in vivo*で膵β細胞量を定量する技術は実用化されていないが，MRI，PETが，将来実用化が期待される侵襲のない，膵β細胞量イメージングの代表的な方法である．OPTは，3D画像によるイメージングの方法として期待が大きい．

〔臨床検査　54：1040-1047，2010〕

(KEYWORDS) 膵β細胞量，アポトーシス，イメージング

糖尿病の病態と膵β細胞量

糖尿病は，主にその成因により分類されている．1型糖尿病は，膵β細胞量の絶対的な減少によるもので，特に1A型では自己免疫学機序による膵β細胞の破壊が病態の主体である．2型糖尿病では，多くの場合，膵β細胞からのインスリン分泌の障害と肝臓や骨格筋などにおけるインスリン抵抗性の両者が認められる．2型糖尿病の病態形成において，インスリン分泌の障害とインスリン抵抗性のどちらが重要であるのかについては，長期間にわたる論争があった．2型糖尿病の発症初期には，高インスリン血症がしばしば認められることより，2型糖尿病では膵β細胞にprimaryな異常はないという考え方があった．しかし，ここ最近の研究成果より，2型糖尿病は，膵β細胞にもともと何らかの障害を有する場合に発症するという考え方が主流となってきている．

Butlerら[1)]は剖検膵組織の検討から，2型糖尿病患者において膵β細胞量が減少していることを報告した(図1)．この報告によれば，空腹時血糖がIGTの領域にある肥満患者の膵β細胞量は，正常血糖の肥満者に比べて少ないことがわかる．つまり，代償的肥大(過形成)を経て，何らかの要因で膵β細胞が減少に転じた個体，あるいは膵β細胞の代償的肥大(過形成)能力がもともと弱い個体において，血糖上昇が起こると考えられる．空腹時血糖が糖尿病領域まで達した個体では，さらに膵β細胞量は小さくなり，非肥満2型糖尿病患者においても膵β細胞量の減少は有意である．英国で行われたUKPDS(U.K. Prospective Diabetes Study)[2)]で，膵β細胞のインスリン分泌能が糖尿病発症の10～12年前から始まっていることが推察されたが，Butlerらの結果は，2型糖尿病発症時すでに膵β細胞の数(容量)の減少も起こっていることを示していると考えられる．

つまり，膵β細胞の容量(膵β細胞量)は，1型糖尿病のみならず，2型糖尿病の発症・進展においても重要な因子であり，インスリン抵抗性があっても，生体の需要に応じて，膵β細胞が自

1）山口大学大学院医学系研究科病態制御内科学・助教
2）同・教授

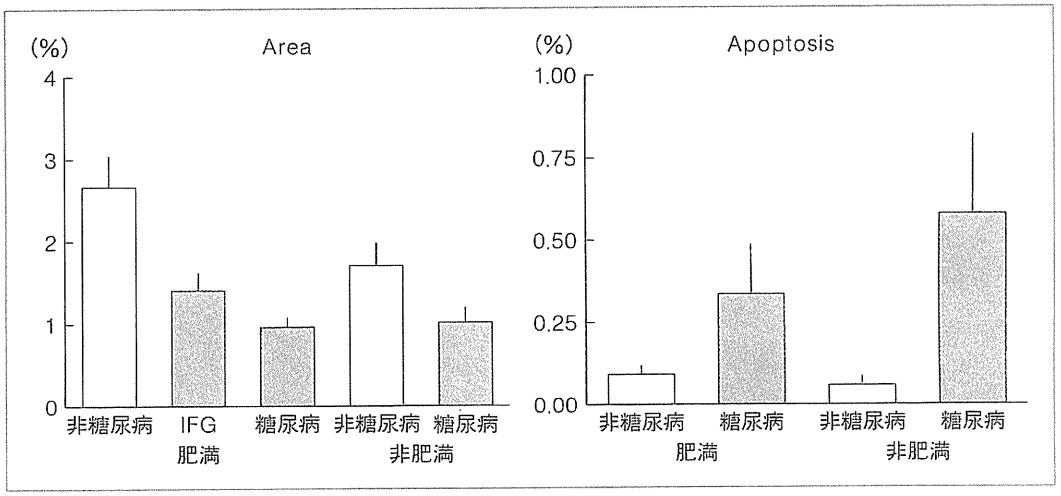

図1　2型糖尿病における膵β細胞の容量(面積比，左)とアポトーシス(右)

〔文献1)より引用〕

らその容量を増大させてインスリンを適切に分泌し続ける間は糖尿病を発症しない．しかし，インスリンの需要に対して見合うだけのインスリンが供給できなくなった状態，膵β細胞の容量を増大させることができなくなった状態で，糖尿病が発症することが推測される．

膵β細胞量と膵β細胞死

膵β細胞量の調節には，①膵β細胞自体の再生，②膵β細胞の大きさ，③膵β細胞の新生，④膵β細胞のアポトーシス，の4つの要素が密接に関連していると考えられている[3]．Butlerらの報告によると，再生と新生には，糖尿病群と非糖尿病群で有意差は認められなかったが，アポトーシスにおいてのみ有意差が認められたため(図1)，アポトーシスがヒトの膵β細胞量の調節には最も重要な因子であることが推察される．しかし，特殊な状況下でない限り，一時期にアポトーシスを起こしている膵β細胞の割合はラ氏島数当たり1%にも満たない．そのため，膵β細胞死(アポトーシス)を生体でリアルタイムに，特にヒトの生体で評価するのは極めて困難であり，現在もなお不可能であると言わざるをえない．

そう考えると，膵β細胞死というものは，残存膵β細胞量で代用して評価するしかないと考えられる．一方で，膵β細胞量を生体内で定量することに関しては，いくつかの優れた知見が得られてきている．そうした状況を踏まえ，膵β細胞死の検査を代用するものとして，(残存)膵β細胞量の定量を中心に概説したい．

In vivo 検査による膵β細胞量の評価

1．動物モデル

膵β細胞死を病理所見以外で評価するのは現時点では不可能であり，その代用として残存膵β細胞量で評価することでさえ実際には容易なことではない．最も多い試みとしては，インスリン分泌能を評価することで，膵β細胞量を推測しようとすることである．

ミニブタを使った研究では，*in vivo* 検査と病理所見から得られた実際の膵β細胞量との関連を見出そうとしている．2003年には，ミニブタをストレプトゾトシン処理することで膵β細胞を死滅させ，インスリン分泌を評価すると，経静脈的グルコース負荷時の急性インスリン分泌応答(acute insulin response；AIR)とBCM(β cell mass)との間に良好な相関($r^2=0.6155$)が得られたことが報告された[4]．

2．HOMA-β(homeostasis model assessment β cell function)，AIR，C-ペプチドによる膵β細胞量の評価

1型糖尿病では，何らかの誘因によって膵β細胞容量が徐々に減少し，残存膵β細胞量が10%前後となったときに糖尿病を発症するとされている[5]．これと同じようなことが，2型糖尿病でも