Table 1. First-phase insulin response (FPIR) in 138 healthy Italian children and adolescents, divided into three groups according to pubertal development

Group	Subjects <i>n</i>	Sex Female/ male	Tanner pubertal stage	FPIR (μ U/ml)											
				range	1 st	3 rd	5 th	10 th	25 th	50 th	75 th	90 th	95 th	97 th	99 th
1	70	20/50	I	53.0–310.0	53.0	61.4	66.6	72.0	93.5	142.1	187.7	223.7	258.0	260.7	310.0
2	41	17/24	II–III	53.6–390.4	53.6	64.5	65.9	91.5	99.1	148.1	202.0	304.0	330.2	358.3	390.4
3	27	10/17	IV–V	76.6–365.1	76.6	76.6	86.0	105.0	126.6	163.5	256.4	324.6	345.2	365.1	365.1

Yours sincerely,

R. Lorini, M. Vanelli on behalf of the Prediabetes Study Group of the Italian Society for Pediatric Endocrinology and Diabetology (SIEDP)*

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Age-dependent association of HLA-A24 in Japanese IDDM patients

Dear Sir,

We were interested to read the recent article by Dr. Awata et al. [1]. However, the authors mentioned only HLA class II age-dependent heterogeneity in Japanese insulin-dependent diabetic (IDDM) patients [1]. It has already been reported that HLA-A24 in Japanese IDDM patients shows a significant association with complete beta-cell destruction, suggesting that A24 promoted pancreatic beta-cell destruction in co-operation with other IDDM-susceptible HLA antigens [2]. Adult-onset IDDM has been reported to show a slower progression

of pancreatic beta-cell destruction with milder symptoms than juvenile-onset IDDM at the time of diagnosis in both Caucasian and Japanese patients [3, 4]. In Japanese patients, while associations of the HLA class II genes with IDDM have been established, little has been reported regarding the possible age-dependency of HLA class I.

A total of 340 unrelated IDDM patients (200 women and 140 men; mean age of diabetes onset 13.8 ± 6.5 years [range 0–30 years]) were randomly selected from out-patients attending the Diabetes Center, Tokyo Women's Medical College (TWMC) and Hokkaido IDDM Treatment Group (HITG), Japan. All the patients were Japanese and were residents of the Tokyo and Hokkaido areas. There are no regional variations in IDDM risk in Japan [5]. The diagnosis of IDDM was made according to the guidelines of the National Diabetes Data Group [6]. In addition, a daily urinary reactive C-peptide value of less than 6.6 nmol/day [2] or anti-glutamic acid decarboxylase (GAD) antibody value of more than 8 U/ml [7] was used to define IDDM. At the time of this study, all the patients

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Table 1. Frequencies of HLA serological types of the subjects studied

n HLA	Age range (years)					pc value vs control group			
	0–5 ^a n (%)	6–12 ¹⁰³ n (%)	13–19 ⁷¹ n (%)	20–29 ⁸⁶ n (%)	control group ¹⁷⁶ n (%)	0–5	6–12	13–19	20–29 (years)
A2	29 (36)	59 (57)	36 (51)	35 (41)	75 (43)	NS	NS	NS	NS
A11	9 (11)	16 (16)	5 (7)	12 (14)	37 (21)	NS	NS	NS	NS
A24	55 (69)	87 (84)	51 (72)	45 (52)	85 (49)	0.023	< 0.0001	0.008	5.401
A26	15 (19)	23 (22)	14 (20)	17 (20)	31 (18)	NS	NS	NS	NS
B7	11 (14)	15 (14)	3 (4)	7 (8)	16 (9)	NS	NS	NS	NS
B35	10 (13)	27 (26)	8 (11)	8 (9)	27 (15)	NS	NS	NS	NS
B44	16 (29)	11 (11)	10 (14)	17 (29)	25 (14)	NS	NS	NS	NS
B54	32 (40)	36 (35)	27 (38)	30 (35)	15 (9)	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cw7	15 (19)	33 (32)	8 (11)	24 (28)	40 (23)	NS	NS	NS	NS
DR2	3 (4)	8 (7)	1 (1)	1 (1)	31 (18)	NS	NS	0.026	0.006
DR4	49 (61)	68 (66)	41 (57)	50 (58)	55 (31)	< 0.0001	< 0.0001	0.002	< 0.0001
DR8	13 (16)	26 (25)	9 (13)	18 (24)	41 (23)	NS	NS	NS	NS
DR9	31 (39)	41 (40)	30 (42)	30 (40)	41 (23)	0.216	0.070	0.058	0.950
DQ3	44 (55)	61 (59)	49 (69)	40 (52)	62 (35)	0.072	< 0.0001	< 0.0001	0.120
DQ4	50 (63)	62 (60)	42 (59)	46 (61)	50 (28)	< 0.0001	< 0.0001	0.0002	< 0.0001

^a The 0–5 year group consisted of 50 from TWMC and 30 from HITG; the 6–12 year group consisted of 81 from TWMC and 22 from HITG; the 13–19 year group, 66 from TWMC and 5

from HITG; all the 20–29 year group belonged to TWMC. The numbers of patients with Graves' disease in the subgroups were 0, 0, 6, and 3, respectively.

were prone to ketosis, and insulin treatment had been started immediately after the onset of IDDM. Their mean body mass index was $20.1 \pm 0.4 \text{ kg/m}^2$. The mean daily insulin dose was $0.98 \pm 0.21 \text{ IU} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$. Of the 340 IDDM patients 9 also had Graves' disease. A normal control group consisted of 176 healthy individuals, who were residents of the Tokyo area. Since the incidence of IDDM in Japanese patients appears to have two peaks of onset, one around the age of 4–5 years and another around the age of 13–14 years [8], the 340 IDDM patients were divided into four groups as shown in Table 1. HLA class I and class II serological types were determined by the standard microcytotoxicity test. Differences were analysed for statistical significance using the two-tailed Fisher's exact test. Corrected *p* values were calculated by multiplication by the number of antigens examined at each locus (10 for A, 20 for B, 10 for C, 20 for DRB1 alleles, and 25 for DR-DQ haplotypes).

Table 1 shows the frequencies of major HLA serological antigens in the four groups and the control group. Among HLA class II, a significant increase of DR4 and DQ4 (of which 90–95 % were typed as DRB1*0405 and DQB1*0401 by DNA typing) was observed in all subgroups (*pc* < 0.0001), while no significant age-dependent variation in the frequencies of DR8 and DR9 was observed among the four subgroups and control subjects. However, for class I antigens, the frequencies of A24 in the 0–5, 6–12, and 13–19 year groups were significantly higher than that in the control group, while the frequency of B54 showed significant increases in all subgroups compared with control subjects (*pc* < 0.0001). The strong linkage disequilibrium among B54, DR4 and DQ4 [9] is considered to be reflected in the increase of these antigens.

These results suggest that certain HLA class I antigens or haplotypes also contribute to age at time of IDDM onset. Moreover, the significant increase of A24 in younger patients may support that the antigens promote pancreatic beta-cell destruction in IDDM at a younger age; however, the exact mechanism is still to be elucidated. At present, it is difficult to interpret the finding that the frequency of A24 in the 0–5 year group was lower than in the 6–12 year group. The development of IDDM in the 0–5 year group may have been due to genetic or congenital factors other than HLA antigens.

Yours sincerely,

M. Mizota, Y. Uchigata, S. Moriyama, K. Tokunaga, N. Matsura, J. Miura, T. Fuji, Y. Omori

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Response from the authors

Dear Sir,

We would like to thank Dr. Mizota et al. for their response to our letter [1]. In our letter, we reported age-dependent heterogeneity of HLA DRB1*0802 and DRB1*0901 (class II) alleles in Japanese insulin-dependent diabetes mellitus (IDDM); the former allele was preferentially increased in childhood (<15 years) onset IDDM, while the latter preferentially increased in adult (>30 years) onset IDDM. Mizota et al. report here a possible age-dependent association of HLA-A24 (class I) in Japanese IDDM patients, but they did not observe age-dependent associations of HLA DR4, DQ4, DR8 and DR9. Although their data appear insufficient for HLA class II alleles in some respects (such as the lack of the DR8 subtypes and the lack of patients with onset later than 30 years), I was interested in their observation regarding HLA-A24. Previously, in Japanese patients, Kobayashi et al. [2] reported associations of the class I allele with acute onset of the disease and with complete beta-cell destruction [3].

To verify their findings, we also determined the HLA-A24 allele in our data set. HLA-A24 was detected by oligonucleotide typing [4] using the probe, A254 (this probe also reacts to HLA-A23, but the frequency of A23 is very low in Japanese subjects). Table 1 shows our results. A preferential increase of A24 in patients with younger age at onset was observed, although A24 was not significantly increased in any age-group compared with the control group. Furthermore, the frequency of A24 was lower in patients with slowly progressive IDDM (defined as >1 year between onset and insulin start) than other fast progressive patients (19/33, 58% vs 70/102, 69%). There is no linkage between A24 and the susceptible class II alleles (DRB1*0405, 0802, 0901) in the patients. These results are generally consistent with the reports by Mizota et al. and Kobayashi et al. [2], and may support that HLA-A24 modulates the HLA class II effect in the development of the disease in Japanese subjects. However we feel that the effect of A24, if it exists, appears weak, and we could not confirm the association

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Table 1. Frequencies of HLA-A24 in Japanese subjects

	Age range (years)					Control
	0-5	6-12	13-19	20-29	30	
<i>n</i>	10	24	29	30	42	171
	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)	<i>n</i> (%)
A24	8 (80)	18 (75)	21 (72)	18 (60)	24 (57)	104 (61)

of A24 with complete beta-cell destruction (defined as reactive C-peptide value <0.033 nmol/l) in our patients. Thus, further studies are necessary to confirm the possible role of HLA-A24. Indeed, a possible contribution of the HLA class I region to IDDM susceptibility is also debatable in Caucasians [5-7].

Yours sincerely,

T. Awata, T. Izumida, C. Kikuchi, S. Katayama

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