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Association of distal chromosome 2q with IDDM in Japanese subjects

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Summary An insulin-dependent diabetes mellitus (IDDM)-susceptibility gene *(IDDM13)* has recently been mapped to a region of distal chromosome 2q, which is syntenic to the region of mouse chromosome 1 containing a murine susceptibility gene for IDDM, *Idd5.* To determine the contribution of this region to IDDM disease susceptibility further and to narrow the region for positional cloning of susceptibility genes, we have studied the association of distal chromosome 2q with IDDM in the genetically distinct Japanese population. A 137 mobility unit (mu) allele at *D2S137* locus was significantly associated with IDDM (odds ratio 1.92, $p = 0.0016$). Other markers, *D2S301* and *D2S143,* located in the same region were not associated with IDDM, indicating that *IDDM13* is in linkage disequilibrium with *D2S137,* but not with *D2S301* or *D2S143.* The association of *D2S137* with IDDM was observed in patients lacking one of two high risk HLA alleles, *DQB1*0303* and *DQB1*0401,* but not in patients with either of these alleles. The frequency of high risk HLA alleles was significantly lower in patients with the susceptible allele at *D2S137,* suggesting that *IDDM13* contributes to IDDM susceptibility in subjects without high risk genotypes at *IDDM1.* Demonstration of allelic association of *D2S137* with IDDM localizes *IDDM13* in the close vicinity (< 2 centiMorgans) of *D2S137,* greatly facilitating fine structure mapping and positional cloning of *IDDM13.* [Diabetologia (1998) 41: 228–232]

Keywords Genetic susceptibility, linkage disequilibrium, association, positional cloning, microsatellite marker.

Insulin-dependent diabetes mellitus (IDDM) is caused by autoimmune destruction of insulin-producing beta cells of the pancreas in genetically susceptible individuals [1]. Inheritance of IDDM is polygenic with a major locus *(IDDM1)* in the major histocompatibility complex (MHC) [2, 3]. Several additional loci have recently been mapped to the human genome by whole genome scanning with random markers [4–8] and/or a candidate gene approach [9–14]. Most reports, however, are based on data in Caucasian populations, and it is yet to be determined whether loci mapped in one population universally contribute to IDDM susceptibility in other populations.

The Japanese population is unique in studies on the genetics of IDDM not only because they are genetically distinct from Caucasian populations, but also because the incidence of IDDM is very low in this population [15]. We have previously reported that two IDDM loci, *IDDM1* in HLA region [15, 16] and *IDDM2* in the insulin gene *(INS)* region [17], both of which were identified by the candidate gene approach, contribute to IDDM susceptibility in Japanese in the same way as in Caucasians. The contribution of other loci, however, to IDDM susceptibility in Japanese is largely unknown due to their low incidence and, as a consequence, the limited number of multiplex families with IDDM.

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In order to fine map and positionally clone susceptibility genes for IDDM, it is necessary to identify markers in linkage disequilibrium with susceptibility genes by demonstrating allelic association of the markers with IDDM. Association of markers with IDDM in genetically distinct populations, such as Japanese and Caucasians, will greatly accelerate fine mapping and positional cloning of susceptibility genes for IDDM because different haplotypes segregate in different ethnic groups [18, 19]. Until recently, association studies were mainly conducted for candidate genes, such as *HLA* and *INS,* but the recent development of fluorescence-based technology for genotyping of microsatellite markers [20] has made it possible to systematically conduct linkage disequilibrium mapping with microsatellite markers even in polygenic diseases, such as IDDM. In particular, markers reported to be linked to IDDM in one population are strong candidates for linkage disequilibrium mapping by association studies in other populations.

Recently, Morahan et al. [21] mapped *IDDM13* to the *D2S137–D2S164* interval on distal chromosome 2q. This region is an important candidate region for IDDM susceptibility not only because susceptibility genes have been mapped to this region in Caucasian populations, but also because this region is syntenic to murine chromosome 1 where a susceptibility gene for IDDM *(Idd5)* was mapped in the nonobese diabetic (NOD) mouse [22, 23]. To study the contribution of this region to IDDM susceptibility further and to narrow the region for subsequent positional cloning of susceptibility genes, we have studied the association of distal chromosome 2q with IDDM in the genetically distinct Japanese population.

Subjects and methods

A total of 319 Japanese subjects (130 patients with IDDM and 189 control subjects) were studied. All patients with IDDM were ketosis-prone and insulin-dependent since diagnosis. Mean ± SD age-at-onset of IDDM was 16.2 ± 13.6 years (range: 3–56 years).

Three microsatellite markers, *D2S137* and *D2S301,* located in the *IDDM13* region [21] and an additional microsatellite, *D2S143,* located proximal to *D2S137* were studied for possible association with IDDM. Genotypes were determined using a fluorescence-based method as reported previously [20, 24]. Briefly, forward PCR primers were labelled with 6-FAM and the PCR products were electrophoresed in 4% denaturing polyacrylamide gel using a Model 373 DNA sequencer (Applied Biosystems, Foster City, Calif., USA) with Genescan 500 ROX (Applied Biosystems, Foster City, Calif. USA) as an internal lane size standard. PCR fragments were sized with Genescan 672 software (Applied Biosystems) genotyped with GENOTYPER software (Applied Biosystems) and alleles were called using a histogram as reported previously.

HLA-*DQA1* and *DQB1* alleles were determined by the PCR-RFLP method as reported previously [16].

Statistical analysis was performed by chi-squared test or Fisher's exact probability test.

Table 1. Allele frequencies of *D2S137* in patients with IDDM and control subjects

Alleles	IDDM patients $n = 260$		Control subjects $n = 378$		Odds ratio	p value
(mu)	n	%	n	%		
135	3	1.2	3	0.8		
137	61	23.5	52	13.8	1.92	0.0016
139	5	1.9	6	1.6		
143	6	2.3	10	2.6		
145	5	1.9	3	0.8		
147	42	16.2	67	17.7		
149	9	3.5	24	6.3		
151	65	25.0	113	29.9		
153	46	17.7	70	18.5		
155	7	2.7	11	2.9		
157	6	2.3	6	1.6		
159	4	1.5	7	1.9		
others[a]	1	0.4	6	1.6		

[a] Rare alleles (frequencies < 0.01)

Table 2. Allele frequencies of *D2S301* in patients with IDDM and control subjects

D2S301 Alleles	IDDM patients $n = 220$		Control subjects $n = 358$	
(mu)	n	%	n	%
220	5	2.3	4	1.1
224	10	4.5	22	6.1
228	8	3.6	7	2.0
230	159	72.3	265	74.0
232	12	5.5	12	3.4
234	4	1.8	14	3.9
238	5	2.3	11	3.1
240	15	6.8	20	5.6
others[a]	2	0.9	3	0.8

[a] Rare alleles (frequencies < 0.01)

Results

The frequency of the 137 mobility unit (mu) allele of *D2S137,* which was reported to be linked to *IDDM13* in Australian subjects, was significantly higher in IDDM patients than in control subjects (Table 1). By contrast, *D2S301,* which was also reported to be linked to IDDM [21], and *D2S143,* which is located opposite *D2S301,* were not associated with IDDM (Table 2), suggesting that *IDDM13* is in linkage disequilibrium with *D2S137,* but not with *D2S301* or *D2S143.*

Because of the well-known contribution of HLA to IDDM susceptibility, the association of *D2S137* was studied relative to HLA genotypes of the subjects. The association of *D2S137* with IDDM was stronger in patients lacking one of two high risk *DQB1* alleles (Table 3). The frequency of *DQA1*0301,* another high risk DQ allele, was too high (> 95%) in IDDM patients to assess the effect of this allele on the association of *D2S137* with IDDM.

Table 3. Association of 137 mu allele at *D2S137* locus with IDDM relative to HLA

			137 mu *n*	allele %	Odds ratio	*p* (vs control)
Control		($n = 378$)	52	13.8		
IDDM	Total	($n = 260$)	61	23.5	1.92	0.0016
	HLA-genotyped	($n = 144$)	35	24.3	2.01	0.0038
	*DQB1*0303* (+)	($n = 50$)	10	20.0	1.57	NS
	(–)	($n = 94$)	25	26.6	2.27	0.0026
	*DQB1*0401* (+)	($n = 84$)	18	21.4	1.71	NS
	(–)	($n = 60$)	17	28.3	2.48	0.003

Table 4. Characteristics of IDDM patients with and without 137 mu allele at *D2S137* locus

	137 mu allele (+) ($n = 55$)	137 mu allele (–) ($n = 75$)	Odds ratio	*p* value
Sex (females)	32 (58.2 %)	49 (65.3 %)	0.74	NS
High risk HLA[a]	24/32 (75 %)	38/40 (95 %)	0.16	0.0175
Age-at-onset[b]	13.9 ± 11.6	17.8 ± 14.6		NS

[a] *DQB1*0303* and/or **0401*
[b] mean ± SD (years old)

To clarify the characteristics of IDDM patients with a susceptibility marker in the *IDDM13* region, the clinical characteristics of IDDM patients with and without 137 mu allele at *D2S137* locus were compared (Table 4). The frequency of high risk HLA alleles was significantly lower in IDDM patients with 137 mu allele than in those without (odds ratio 0.16, $p = 0.0175$). Since linkage of *D2S137* with IDDM was reported to be stronger in families with affected females [21], the frequency of female patients was compared between patients with and without the 137 mu allele. In contrast to the previous study, the frequency of female patients was not higher, but rather lower, in patients with the 137 mu allele than in those without. There was no significant difference in age-at-onset of IDDM between the two groups (Table 4).

Discussion

Distal chromosome 2q is an important candidate region for IDDM susceptibility not only because several susceptibility genes have been mapped to this region in Caucasian populations [14, 21, 25, 26], but also because this region is syntenic to murine chromosome 1, where a susceptibility gene for IDDM was mapped in the NOD mouse [22, 23]. Recently, Morahan et al. [21] mapped a susceptibility gene for IDDM *(IDDM13)* to the *D2S137–D2S164* interval on chromosome 2q. To study whether or not the contribution of this region to IDDM susceptibility is universal and to fine map the susceptibility gene in this region, we studied the association of markers in this region with IDDM in the genetically distinct Japanese population. One allele at *D2S137* locus was associated with IDDM, suggesting that chromosome 2q contains a susceptibility gene for IDDM in Japanese as well as in Caucasians, and that *D2S137* is in linkage disequilibrium with *IDDM13.* No association was observed with *D2S301,* another marker in the same interval, and *D2S143* located proximal to the *IDDM13* region, suggesting that *IDDM13* is located close to (< 2cM) *D2S137,* but not to *D2S301* or *D2S143. D2S301* and *D2S143* are located approximately 4cM distal and 1cM proximal, respectively, to *D2S137,* suggesting that *IDDM13* is located in the less than 5cM interval between *D2S301* and *D2S143. IGFBP5,* which encodes insulin-like growth factor-binding protein 5 and was suggested to be a candidate gene in a previous report [21], is located in the *D2S301–D2S143* interval, and therefore is still a candidate for *IDDM13.*

Because of the well-known contribution of HLA to IDDM susceptibility, it is important to study the effect of non-HLA susceptibility genes relative to HLA genotypes. In fact, linkage of *IDDM13* markers with IDDM was reported to be much stronger in sibpairs who do not share HLA alleles [21]. Our study in the genetically distinct Japanese population confirmed the previous findings in that the association of *D2S137* with IDDM was stronger in patients who lacked one of two susceptible HLA alleles, and the frequency of disease-associated alleles at the HLA-*DQB1* locus was significantly lower in patients with the disease-associated allele at the *D2S137* locus. These data suggest that *IDDM13* contributes to IDDM susceptibility in subjects without high risk genotypes at *IDDM1* and this effect is universal. The Japanese population is unique in that the incidence of IDDM is very low as compared with Caucasian populations [15]. This may explain why we could detect a significant effect of *IDDM13* in a case-control

study, because the effect of *IDDM13* appeared to be evident in subjects without strong susceptibility genes at other loci such as *IDDM1,* and therefore, its contribution of IDDM susceptibility may be easier to detect in low incidence populations where strong susceptibility genes are expected to be less common as compared with Caucasian populations. This suggests the possibility that populations with a low incidence of IDDM, such as Japanese, may serve as useful samples to demonstrate the effect of non-HLA genes on IDDM susceptibility.

In addition to *IDDM13, IDDM7* and *IDDM12* have also been mapped to distal chromosome 2q [14, 25, 26]. *IDDM7* was reported to be linked to, and associated with, *D2S152* on chromosome 2q31–q33 in Italian, Sardinian and United States populations, but not in United Kingdom and Danish populations [25]. An adjacent, but distinct marker, *HOXD8,* was reported to be linked to IDDM in Caucasian families in the United States and United Kingdom [26]. *IDDM12* was reported to be linked to and associated with *CTLA4* on chromosome 2q33 in Spanish and Italian populations, but not in United Kingdom, United States and Sardinian populations [14]. We have previously studied the possible association of *D2S152* with IDDM in Japanese, but only a very weak association was observed [24]. No significant association was observed for *HOXD8* and *CTLA4* in our preliminary study in Japanese (data not shown). These data further emphasize the importance of genetics studies in different populations. Due to the multifactorial nature of the disease, the contribution of some loci, such as *IDDM1* and *IDDM2,* was repeatedly shown among diverse populations, but the contribution of other loci, such as *IDDM7* and *IDDM12,* was detected in some but not in other populations. In any case, detection of linkage disequilibrium between a marker and a susceptibility gene in some populations is an essential step for positional cloning of the disease gene. Markers located in intervals where IDDM susceptibility genes were mapped in one population should therefore be tested for possible association with IDDM in other populations. Fluorescence-based technology for genotyping of microsatellite markers [20] has made it possible to conduct such association studies systematically as shown in this and other studies [14, 25, 27]. Demonstration of allelic association of *D2S137* with IDDM localizes *IDDM13* in the close vicinity (< 2 centiMorgans) of *D2S137,* greatly facilitating fine structure mapping and positional cloning of *IDDM13.*

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