

# Capture of Type 1 Diabetes–Susceptible HLA DR-DQ Haplotypes in Japanese Subjects Using a Tag Single Nucleotide Polymorphism

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**OBJECTIVE** — To identify type 1 diabetes–susceptible HLA DR-DQ haplotypes using tag single nucleotide polymorphisms (SNPs) and to estimate the disease risk using these tag SNPs.

**RESEARCH DESIGN AND METHODS** — Five tag SNPs were typed in a total of 211 Japanese subjects including 201 patients with type 1 diabetes who had already been typed for HLA-DRB1, -DQA1, and -DQB1 alleles and 300 control subjects.

**RESULTS** — Tag SNP rs2395185 captured haplotypes involving all DR4 specificities and DR9 specificity with a sensitivity of 98.5% and specificity of 94.9%. Using the T allele of rs2395185, we obtained an odds ratio (95% CI) of 2.87 (2.21–3.74) for type 1 diabetes. In addition, rs3129888 captured haplotypes involving HLA-DRB1\*0802 with a sensitivity of 92.3% and specificity of 98.9%.

**CONCLUSIONS** — Typing of two tag SNPs (rs2395185 and rs3129888) may be useful for the screening of Japanese subjects at genetic risk of type 1 diabetes.

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Although the major histocompatibility complex class II region confers susceptibility to type 1 diabetes most strongly (1), DNA typing of the HLA-DR and -DQ alleles is complicated. We attempted to identify type 1 diabetes–susceptible HLA DR-DQ haplotypes rapidly using tag single nucleotide polymorphisms (SNPs) and further estimated the disease risk using these tag SNPs.

## RESEARCH DESIGN AND METHODS

The tag SNPs for HLA class II alleles were typed in a total of 211 subjects including 201 patients with type 1 diabetes (115 male subjects and 86 female subjects; mean  $\pm$  SD age at onset  $34 \pm 14$  years) and 10 patients with other diseases (one patient with type 2 diabetes and nine patients with chronic hepatitis C) who had already been typed for HLA-

DRB1, -DQA1, and -DQB1 alleles (2–4) and 300 control subjects (231 male subjects and 69 female subjects).

Based on the study by de Bakker et al. (5), we chose rs2395185 (T) and rs411326 (C) for HLA-DRB1\*0405, rs3129888 (G) for HLA-DRB1\*0802, rs6457617 (T) for HLA-DQA1\*03, and rs3998159 (A) for HLA-DQB1\*0303 as the tag SNPs. These SNPs were genotyped using TaqMan Assays (Applied Biosystems, Foster City, CA). This study was approved by the Committee for Investigations Involving Human Subjects of Toranomon Hospital.

We performed two-by-two contingency analysis (Fisher exact probability test) and determined the sensitivity and specificity of tag SNPs for identifying specific DRB1-DQA1-DQB1 haplotypes. In the case-control study, the odds ratios (ORs) for developing type 1 diabetes and

the 95% CIs were calculated by logistic regression.

**RESULTS** — As for rs2395185, all (18/18) subjects who had two copies of haplotypes involving DRB1\*0405, which were referred to as haplogenotype 0405/0405, had the TT genotype. Only 64.1% (59/92) of the subjects who had one copy of the haplotype involving DRB1\*0405 and the other copy of the haplotype not involving DRB1\*0405 (referred to as haplogenotype 0405/X) had the GT genotype, and only 23.8% (24/101) of the subjects who had two copies of haplotypes not involving DRB1\*0405 (referred to as haplogenotype X/X) had the GG genotype (Fig. 1A). The sensitivity for rs2395185 to capture haplotypes involving DRB1\*0405 was 98.4% (125/128), but the specificity was only 51.0% (150/294). However, interestingly, in the subjects with genotype TT of rs2395185 and haplogenotype 0405/X, all (31/31) X haplotypes corresponded to those involving DR4 or DR9 specificities (Fig. 1A). Here, DR4 specificities included DRB1\*0401, 0403, 0404, 0405, 0406, 0407, and DR9 specificity included DRB1\*0901. Furthermore, in the subjects with genotype TT of rs2395185 and haplogenotype X/X, 97% (70/72) of the T alleles of rs2395185 linked up with haplotypes involving DR4 or DR9 specificities (Fig. 1A). In contrast, in the subjects with genotype GG of rs2395185 and haplogenotype X/X, 98% (47/48) of the G allele of rs2395185 linked up with haplotypes not involving DR4 or DR9 specificities (Fig. 1A). Given such an allocation of rs2395185 to DR4 or DR9 specificities, in the subjects with the GT genotype of rs2395185 and haplogenotype X/X, 88% (36/41) of the T allele of rs2395185 linked up with haplotypes involving DR4 or DR9 specificities. Thus, in all subjects analyzed, rs2395185 captured haplotypes involving all DR4 specificities and DR9 specificity with a sensitivity of 98.5% (262/266) and specificity of 94.9% (148/156) ( $P < 0.0001$ ) (supplemental Fig. 1, available in an online appendix at <http://care.diabetesjournals.org/cgi/content/full/dc09-1210/DC1>).

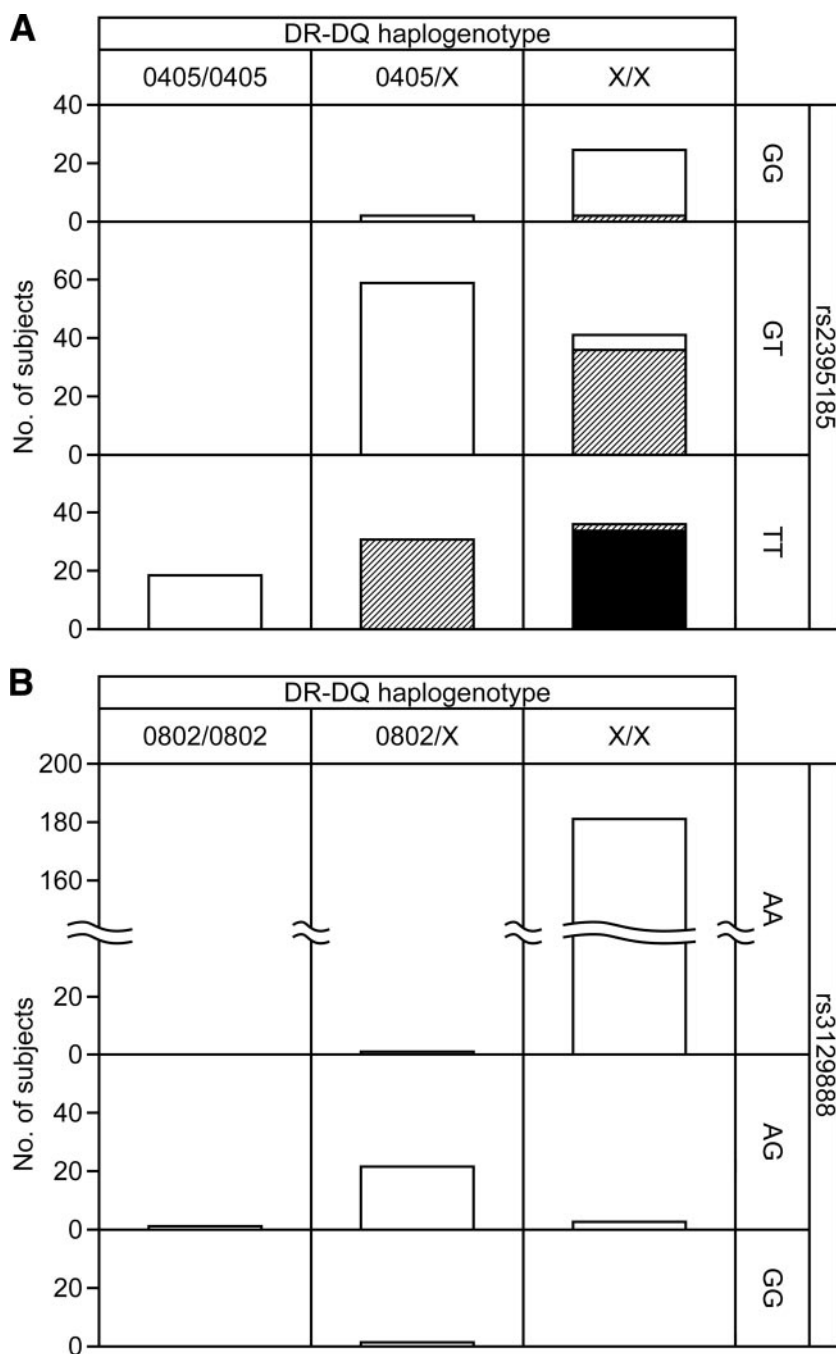
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**Figure 1**—Relationships between genotypes of rs2395185 and haplotype combinations of those involving HLA-DRB1\*0405 (0405) or not (X) (A), and genotypes of rs3129888 and haplotype combinations of those involving HLA-DRB1\*0802 (0802) or not (X) (B). In panel A, diagonally lined portions in the bar indicate that haplotype X (one of X haplotypes in the case of X/X) involves DR4 or DR9, and the portion colored black in the bar indicates that haplogenotype X/X consists of two copies of haplotypes involving DR4 or DR9.

In addition, 24 of 26 haplotypes involving DRB1\*0802 had G allele of rs3129888 (sensitivity 92.3%), and 390 of 394 haplotypes not involving DRB1\*0802 had A allele of rs3129888 (specificity 98.9%) ( $P < 0.0001$ ) (Fig. 1B).

As for the remaining tag SNPs, sensitivities for tagging corresponding HLA al-

leles were high (>90%), but specificities were not adequate (17.4–64.4%) (supplemental Fig. 2). Unlike rs2395185, rs411326 could not capture haplotypes involving DR4 or DR9 specificities (sensitivity 69.3% and specificity 74.3%) (supplemental Fig. 2A).

Using the T allele of rs2395185, we

obtained OR 2.87 (95% CI 2.21–3.74) for type 1 diabetes. The ORs for type 1 diabetes in the GT genotype or the TT genotype of rs2395185 were 2.72 (1.65–4.62) or 9.49 (5.37–17.3), respectively. On the other hand, the distribution of alleles or genotypes of rs3129888 did not differ between patients with type 1 diabetes and control subjects (supplemental Table 1).

**CONCLUSIONS**— Capture of haplotypes involving all DR4 specificities and DR9 specificity by rs2395185 may be due to the involvement of both DR4 and DR9 in the DR53-associated antigens (6). The haplotypes that rs2395185 captured were DRB1\*0405-DQA1\*03-DQB1\*0401, 0402, or 0302, DRB1\*0403, 0404, 0406, or 0407-DQA1\*03-DQB1\*0302, and DRB1\*0901-DQA1\*03-DQB1\*0303 (supplemental Fig. 1). In addition to the haplotypes of DRB1\*0405-DQA1\*03-DQB1\*0401 and DRB1\*0901-DQA1\*03-DQB1\*0303 (7–10), the haplotype of DRB1\*0405-DQA1\*03-DQB1\*0402 and DRB1\*0405-DQA1\*03-DQB1\*0302 also indicates susceptibility in the Japanese subjects (9,10). Although the haplotypes of DRB1\*04-DQA1\*03-DQB1\*0302 were neutral or protective when DRB1\*04 was other than \*0405 (7–9), these haplotypes, especially DRB1\*0403 or 0406-DQA1\*03-DQB1\*0302, formed the susceptible DR3/4-DQB1\*0302 genotype in Korean and Taiwanese subjects (11). The haplotype of DRB1\*0401-DQA1\*03-DQB1\*0301 did not produce susceptibility (7,8), but the frequency of these haplotypes was relatively low (1.13%) (12). Thus, rs2395185 was able to capture all type 1 diabetes-susceptible or potentially susceptible DR-DQ haplotypes except for DRB1\*0802-DQA1\*03-DQB1\*0302 (7–8). In fact, the T allele of rs2395185 provided a high OR (2.87) for the risk of type 1 diabetes, which was almost equivalent to that of haplotype of DRB1\*0405-DQB1\*0401 (2.9–3.88) or haplotype of DRB1\*0901-DQB1\*0303 (2.54–4.34) in another study in Japanese subjects (8). Haplotype of DRB1\*0802-DQA1\*03-DQB1\*0302 could be captured by rs3129888. Although, in this study, allele frequency of DRB1\*0802 was less than that in the previous study (9), it may be due to older onset age of diabetes in our patients (8).

In Caucasians, a two-SNP test for screening the risk genotype DR3/4-DQ8 was reported recently (13). In Japanese

subjects, two tag SNPs of rs2395185 and rs3129888 captured different susceptible DR-DQ haplotypes. Therefore, simultaneous typing of these two SNPs will facilitate the screening of subjects at risk for type 1 diabetes in the Japanese population, especially in the patients with type 2 diabetes because ~10% of them are positive for at least one autoantibody to islet cells and progress to an insulin-dependent state (slowly progressive type 1 diabetes) (14).

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