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Genome-wide linkage analysis of type 2 diabetes mellitus reconfirms the susceptibility locus on 11p13–p12 in Japanese

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Abstract Type 2 diabetes mellitus is a heterogeneous disorder, and the development of type 2 diabetes mellitus is associated with both insulin secretion defect and insulin resistance. The primary metabolic defect leading to type 2 diabetes mellitus has been thought to be varied among populations, especially in Japanese and Caucasians. Here, we have done the genome-wide scan for type 2 diabetes mellitus using 102 affected Japanese sib-pairs to identify the genetic factors predisposing to type 2 diabetes mellitus. Nonparametric linkage analysis showed one suggestive evidence for linkage to 11p13–

p12 [*D11S905*: two-point maximum LOD score (MLS) of 2.89 and multipoint MLS of 2.32] and one nominally significant evidence for linkage to 6q15–q16 (*D6S462*: two-point MLS of 2.02). Interestingly, the 11p13–p12 region was reported to be a susceptibility locus for Japanese type 2 diabetes mellitus with suggestive evidence of linkage, and *D11S905* was within 5 cM to *D11S935* with the highest MLS in the previous linkage analysis reported. The only overlapped susceptibility region with suggestive evidence of linkage for Japanese type 2 diabetes mellitus was *D11S935–D11S905* among

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the three reports including this study. These results taken together suggest that a susceptibility gene for type 2 diabetes mellitus in Japanese will reside in 11p13–p12.

Keywords Type 2 diabetes mellitus · Japanese · Affected sib-pairs · Linkage · Chromosome 11p

Introduction

Type 2 diabetes mellitus is one of the most common diseases, and its prevalence is dramatically increasing worldwide (Zimmet et al. 2001). Type 2 diabetes mellitus is a heterogeneous disorder, and the development of type 2 diabetes mellitus is associated with both insulin secretion defect and insulin resistance. Japanese patients with type 2 diabetes mellitus were reported to be characterized by a lower body mass index (BMI) and lower fasting insulin levels than other populations (Ehm et al. 2000). Insulin secretion defect is thought to be the primary defect in Japanese (Kadowaki et al. 1984) whereas impaired insulin sensitivity is the first metabolic defect predisposing to the development of type 2 diabetes mellitus in Caucasians (Martin et al. 1992). These findings suggest that Japanese individuals with type 2 diabetes mellitus will have a different genetic risk factor, which affects the responsiveness of insulin secretion to glucose, from other populations. Therefore, we need to identify the susceptibility genes for the development of type 2 diabetes mellitus in Japanese to start a primary prevention based on genetic information and to develop the personalized medicine for type 2 diabetes mellitus in Japanese. So far, two whole-genome linkage analyses were carried out using 224 affected sib-pairs (ASPs) from 159 Japanese families (Mori et al. 2002) and 256 ASPs from 164 Japanese families (Iwasaki et al. 2003), besides the analysis of 45 ASPs from 18 Japanese American families (Ehm et al. 2000). The Japanese people may have advantages in the genetic analysis of polygenic disorders like diabetes since they are supposed to be a relatively homogeneous population. However, the two previous reports on the ASP analysis in Japanese did not give good overlapping regions, except for 6p and 2q, and it has been argued that the replication by the third panel is indispensable for genetic susceptibility loci in Japanese. Here, we have carried out the third whole-genome linkage analysis on 102 ASPs from 102 Japanese families to identify the susceptibility loci for the development of type 2 diabetes mellitus.

Subjects and methods

One hundred and two ASPs with type 2 diabetes mellitus from 102 families were collected mainly from the Kyushu region in southwestern Japan. Parents and other siblings were not available in this study. The participants were interviewed and examined and gave written informed consent. This project was approved by the ethics committees of the related institutes. The diagnosis of type 2 diabetes mellitus was made based on the American Diabetes Association's 1997 criteria (Expert

Fig. 1 Multipoint LOD score map of type 2 diabetes mellitus by linkage analysis of 382 markers in 102 affected sib-pairs. The *horizontal axis* is cM position from the p-terminal end of the chromosome

Fig. 1 (Continued)

Committee on the Diagnosis and Classification of Diabetes Mellitus 1997).

Genomic DNA was isolated from peripheral blood cells using QIAamp DNA Blood Midi Kits (Qiagen). Autosomal whole-genome screening of 382 microsatellite markers (ABI PRISM Linkage Mapping Set Version 2.5-MD10) was performed using an ABI 3730 automatic sequencer (Applied Biosystems). Analyses and assignment of the marker alleles were done with ABI PRISM GeneMapper Software Version 3.0, and 376 markers were available for the linkage analysis. Non-parametric two-point and multipoint linkage analyses

Table 1 Results of linkage analysis of type 2 diabetes mellitus and markers showing evidence of linkage

Marker	cM ^a	Analysis	MLS	P
<i>D6S462</i>	89	Two-point	2.02	0.0097
<i>D11S905</i>	54	Two-point	2.89	0.0013
		Multipoint	2.32	0.0048

^aThe distance of the marker from the p-terminal end of the chromosome in cM

were performed with the MAPMAKER/SIBS program (Kruglyak and Lander 1995), as described (Sakai et al. 2001). Heterozygosities of the markers were estimated

with Merlin program (Abecasis et al. 2002) for all individuals.

Results and discussion

Whole autosomal genome linkage analysis using the ASP method with 382 microsatellite markers was carried out on 102 Japanese ASPs with type 2 diabetes mellitus. In this study, the average heterozygosity of the markers used was 0.72. Multipoint linkage analysis at all autosomal chromosomes using the MAPMAKER/SIBS program revealed only one region on chromosome 11p where the MLS was > 1 (Fig. 1). The highest multipoint MLS was 2.32 ($P=0.0048$) at *D11S905* (Fig. 1, Table 1). On the other hand, two-point linkage analysis revealed two markers, *D11S905* (MLS=2.89, $P=0.0013$) and *D6S462* (MLS=2.02, $P=0.0097$), with evidence of linkage to type 2 diabetes mellitus (Table 1). Although the heterozygosity of *D11S905* was 0.30 in this study, it was 0.75 and 0.60 in our two reports using ASPs (Sakai et al. 2001; Aoki et al. 2004), indicating that *D11S905* itself will be useful in the genetic analysis in terms of heterozygosity in the Japanese population, and particular alleles of *D11S905* might be associated with type 2 diabetes mellitus. The 11p13–p12 region was reported to be linked to Japanese type 2 diabetes mellitus specifically, in which multipoint analysis showed the highest MLS of 3.08 near *D11S935* (Mori et al. 2002). The distance between *D11S905* and *D11S935* is about 5 cM. These findings together suggest that the 11p13–p12 region will be a susceptibility region for Japanese type 2 diabetes mellitus.

In addition to *D11S905*, one nominally significant evidence of linkage was detected at *D6S462* (MLS of 2.02) by two-point analysis. However, the multipoint MLS at *D6S462* was 0.08, and two other reports did not show evidence of linkage to this region (Mori et al. 2002; Iwasaki et al. 2003), suggesting that 6q15–q16 might not be a susceptibility region for type 2 diabetes mellitus. Two susceptibility regions for type 2 diabetes mellitus in Japanese, chromosome 2 (236.8 cM) and chromosome 6 (42.2 cM), were reported to be overlapped between the two previous linkage studies (Iwasaki et al. 2003). However, the MLS at these two loci were < 1 (Iwasaki et al. 2003). Among the three reports including this study, the overlapped susceptibility region with suggestive evidence of linkage for Japanese type 2 diabetes mellitus was *D11S935*–*D11S905* only.

In conclusion, we have reconfirmed that the evidence of linkage for type 2 diabetes mellitus in Japanese to 11p13–p12 and 11p13–p12 will be a promising region for future studies on identification of susceptibility genes for type 2 diabetes mellitus in Japanese.

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Appendix

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