ORIGINAL INVESTIGATION

Association study on chromosome 20q11.21-13.13 locus and its contribution to type 2 diabetes susceptibility in Japanese

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Abstract Several linkage studies have predicted that human chromosome 20q is closely related to type 2 diabetes, but there is no clear evidence that certain variant(s) or gene(s) have strong effects on the disease within this region. To examine disease susceptibility variant in Japanese, verified SNPs from the databases, with a minor allele frequency larger than 0.15, were selected at 10-kb intervals across a 19.31-Mb region (20q11.21-13.13), which contained 291 genes, including hepatocyte nuclear factor 4α (HNF4 α). As a result, a total of 1,147 SNPs were genotyped with TaqMan assay using 1,818 Japanese samples. By searching for HNF4 α

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H. Kato Haplopharma Inc., 2-2-1, Yaesu, Chuo-ku, Tokyo 104-0028, Japan as a representative disease-susceptible gene, no variants of HNF4 α were strongly associated with disease. To identify other genetic variant related with disease, we designed an extensive two-stage association study (725 first and 1,093 second test samples). Although SNP1146 (rs220076) was selected as a landmark within the 19.31 Mb region, the magnitude of the nominal Pvalue (P = 0.0023) was rather weak. Subsequently, a haplotype-based association study showed that two common haplotypes were weakly associated with disease. All of these tests resulted in non-significance after adjusting for Bonferroni's correction and the false discovery rate to control for the impact of multiple testing. Contrary to the initial expectations, we could not conclude that certain SNPs had a major effect on this promising locus within the framework presented

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T. Miyamoto · H. Shiota Department of Ophthalmology and Visual Neuroscience, Institute for Health Biosciences, The University of Tokushima, 3-18-15, Kuramoto-cho, Tokushima 770-8503, Japan here. As a way to extend our observations, we emphasize the importance of a subsequent association study including replication and/or meta-analysis in multiple populations.

Abbreviations

FUSION	The Finland-United States Investigation of
	Non-insulin-dependent diabetes mellitus
	genetics
UTR	Untranslated region
JPT	Japanese in Tokyo
CHB	Han Chinese in Beijing
CEU	Utah residents with ancestry from northern
	and western Europe by the Centre d'Etude

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Introduction

Single-nucleotide polymorphisms (SNPs) are the most abundant form of DNA variation in the human genome. To date, more than one million SNPs have been intensively searched and submitted to public databases as common variants in the genome sequence (Altshuler et al. 2005). Numerous resources of dense SNPs provide a research framework that can be exploited to search for genetic factors relevant to common complex diseases with an association study.

Genetic association studies are mainly implemented using two approaches. On the basis of hypothesis-driven theory, candidate gene analysis is directly connected with systemic knowledge of biological function, whereas a whole-genome association study theoretically investigates the entire genome simultaneously in an unbiased manner (Wang et al. 2005; Hirschhorn et al. 2005). However, no method to analyze the entire genome for complex diseases has been established with respect to the availability of unbiased reliability and cost efficiency (Wang et al. 2005; Hirschhorn and Daly 2005). To improve these methodological problems, we have developed a practical and efficient strategy for an extensive region-wide association study with evenly spaced dense SNPs markers (one marker per 10 kb). This research strategy has been applied to the discovery of disease-causing gene(s) and variant(s) as an effective method (Hamada et al. 2005; Kato et al. 2006).

Type 2 diabetes is a complex metabolic disorder characterized by impaired insulin production, insulin resistance in peripheral tissues, and abnormal glucose output by the liver. It is proposed that multiple genetic and environmental factors contribute to the disease (Permutt et al. 2005; O'Rahilly et al. 2005). Wholegenome linkage studies in families with affected individuals have been undertaken in different ethnic groups, and some mapped loci have been observed. In particular, the suggested linkages overlap on the chromosome 20q locus in Caucasian, African-American, and Asian populations (Ghosh et al. 1999, 2000; Vionnet et al. 2000; Permutt et al. 2001; Duggirala et al. 2001; Luo et al. 2001; Mori et al. 2002; Iwasaki et al. 2003). This region harbors the hepatocyte nuclear factor 4α (HNF4 α) gene, mutations of which cause maturity-onset diabetes of the young (MODY) type 1, a dominantly inherited, and early-onset form of type 2 diabetes (OMIM 12850). It is not yet clear whether common variants in the same gene also have similar effects on late-onset forms of type 2 diabetes. Hence, several association studies have further focused on this gene, especially in Caucasians (Winckler et al. 2005; Bagwell et al. 2005; Silander et al. 2004; Love Gregory et al. 2004; Vaxillaire et al. 2005). Until now, however, there has been no clear evidence on chromosome 20q as to whether susceptibility gene(s) or variant(s) definitively contribute to type 2 diabetes in any populations including Japanese.

In this study, we closely examined SNPs within HNF4 α as a likely candidate gene for common type 2 diabetes with an association study, using 1,818 Japanese samples. Simultaneously, to prevent gaps when searching for genetic variants on chromosome 20q, verified SNPs were extensively examined with a two-stage association study across 19.31 Mb on a putative candidate locus. We studied the contribution of detected variants statistically and extended the knowledge about type 2 diabetes on this important locus.

Materials and methods

Selection of samples for the association study

We prepared two independent samples from 1,818 Japanese individuals (925 cases and 893 controls). The first test samples (725 samples) consisted of 367 type 2 diabetes cases and 358 healthy controls. Next, we repeated the association test with the second test samples (1,093 samples) of 558 type 2 diabetes subjects and 535 controls. A description of the case and control samples is provided in Table 1.

Type 2 diabetes patients were mainly recruited at the Tokushima University Hospital, Kyoto Prefectural Table 1 Clinical

characteristics of 1,818 Japanese samples in the association study (893 controls and 925 cases)

Phenotype	First test sar (725 samples	nples s)	Second test (1,093 sampl	samples les)
	Cases	Controls	Cases	Controls
Number of samples	367	358	558	535
Gender (male/female)	191/176	145/213	277/281	291/244

 38.1 ± 14.2

 21.9 ± 2.9

 4.7 ± 0.3

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 $64.1\,\pm\,10.1$

 23.6 ± 3.4

 7.4 ± 1.4

178 (48.5)

187(51.0)

132 (36.0)

2(0.5)

Age, BMI, and HbAlc are represented as means \pm SD. BMI denotes body mass index

University Hospital, and its related hospitals. The diagnosis was based on medical records using the criteria of the World Health Organization (World Health Organization 1985). Type 2 diabetes was clinically defined as gradual adult onset of the disease with medication. Patients with mitochondrial disease or MODY were completely excluded. Control subjects consisted of members of the general population. A large number of control samples (56.7%) were collected by the Pharma SNP consortium (PSC). This consortium works in close collaboration with the National Millennium Project in Japan. Through this consortium, their health conditions and negative family history of diabetes were thoroughly checked. On examining their birthplace information, all subjects had full Japanese ancestry. Hence, the bias of population stratification caused by different ethnic groups was supposed to be low.

Age (years) BMI (kg/m²)

HbAlc (%)

Age at onset < 50 years (%)

> 50 years (%)

Unknown (%)

Positive family history

(first degree relatives, %)

Genomic DNA was prepared from peripheral blood leukocytes or from Epstein-Barr virus-immortalized lymphoblast cell lines with a standard protocol. The study protocol was approved by the Institutional Review Board of The University of Tokushima and was in accordance with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to blood sampling. All personal identifiers associated with medical information and blood samples were carefully eliminated and replaced with anonymous identifiers in each recruiting institution.

Previous linkage reports

Initially, we examined several published genome-wide linkage studies related to chromosome 20q with multiple ethnic groups and extracted nine representative reports in Supplementary Table 1. To date, for Japanese studies, there have been only three linkage reports, including two positive and one negative report on chromosome 20q after searching the PubMed database.

 62.9 ± 10.0

 23.4 ± 3.3

 7.3 ± 1.3

275 (49.3)

281 (50.4)

224 (40.1)

2(0.3)

One positive report identified nominal evidence of linkage with a maximum LOD (logarithm of odds) score of 1.34 for D20S107 (Mori et al. 2002). Alternative linkage analysis also indicated the same region with a maximum LOD score of 1.99 for D20S107 (Iwasaki et al. 2003). In this region, linkage peak has been reported, but this was confusing because the linkage peak was quite broad and rather low. Another recent report failed to confirm the previous two items of linkage evidence on chromosome 20q (Nawata et al. 2004).

SNPs markers selection

Using the previous positive reports, the linkage position was used to adjust the genetic markers to the reference Marshfield Genetic Map. The linkage peaks confirmed that the putative disease-susceptibility locus was mapped to the 50.81–75.01 cM region on chromosome 20q11.21-13.13, corresponding to a 19.31-Mb interval between STS (sequence-tagged site) markers of D20S195 and D20S196. We selected the 19.31-Mb interval as the target region for the extensive association study (Table 2).

To establish a useful marker set for the association study, we searched SNPs located in the interval between D20S195 and D20S196 in public databases [Database of Single-Nucleotide Polymorphisms (dbSNP) and the SNP Consortium] and Celera Discovery System database. (Celera Discovery System database is no longer in use. It was commercially available until June 2005.) Among the numerous candidate SNPs identified, we selected SNPs that mainly

 37.9 ± 11.4

 22.3 ± 2.9

 4.9 ± 0.3

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Target region on chromosome 20q	Results
Cytobands	20qll.21-20ql3.13
STS marker	D20S195-D20S196
Total target size (bp)	19,310,566
NCBI build 35 contig start position (bp)	29,871,045
NCBI build 35 contig end position (bp)	49,181,611
(A) Size of gene-centric region	13,432,173
$(\text{gene} \pm 10 \text{ kb}) \text{ (bp)}$	
(B) Size of gene-centric region	10,100,670
$(\text{gene} \pm 0 \text{ kb}) (\text{bp})$	
Number of RefSeq genes	291
Number of SNPs	1,147
Number of RefSeq genes	219 (75.3%)
with > 1 SNP (%)	
Average SNPs distance (bp) per (A)	10,820
Average SNPs distance (bp) per (B)	8,797

Table 2 Summary of target region on chromosome 20q, and SNPs markers

Gene information and position are based on the NCBI Build 35 human genome

matched the following criteria: (1) dense and evenly spaced SNPs in both coding and non-coding regions, (2) in the coding region, SNPs particularly placed on the gene locus spanning 10 kb upstream of the transcription start site and 10 kb downstream from the end of the last exon, (3) SNPs suitable for developing a TaqMan allele discrimination assay, and (4) to eliminate the effect of rare variants, which were underrepresented in the database, common SNPs that showed minor allele frequencies (MAF) > 0.15 in 46 healthy unrelated Japanese controls (24 males and 22 females) initially tested (Hamada et al. 2005; Kato et al. 2006).

SNPs genotyping

TaqMan MGB (minor groove binder) probe and primer sets were designed by Applied Biosystems (Applied Biosystems, Foster City, CA). We genotyped SNPs with either TaqMan Universal Master Mix (Applied Biosystems) or a QuantiTect Probe polymerase chain reaction (PCR) kit (Qiagen, Stanford, CA) and 5 ng of DNA, 450 nM of each primer, and 100 nM of probe. PCR conditions were as follows: 95°C for 10 min for enzyme activation, followed by 40 cycles of 92°C or 94°C for 15 s and 60°C for 1 min. Thermal cycling was conducted on an ABI GeneAmp PCR System 9700 (Applied Biosystems). Each 384well plate contained 380 samples of unknown genotypes and four samples of no-template controls. After reaction, the fluorescence of VIC and FAM dyes was measured with an ABI Prism 7900HT using Sequence Detector System (SDS) ver 2.1 software (both from Applied Biosystems).

Data review and quality control criteria to genotyping

In genotyping with the TaqMan allele discrimination assay, genotype calls were identified by clustering the fluorescence intensity measurements for each SNP. Therefore, quality control was assessed by the reliability of the intensity measurements (Hinds et al. 2005).

We first checked the number of observed genotyped clusters and eliminated SNPs that could not be clustered or for which our call rate was lower than 98%. Expected genotyping accuracies were estimated with the quality score algorithm used in SDS ver 2.1 program (Applied Biosystems). The intensity measurements were carefully checked for accuracy by two independent researchers. In addition, the deviation of genotype distribution was checked by the Hardy– Weinberg equilibrium test. The test for deviation from Hardy–Weinberg equilibrium was quite effective for identifying artifacts and improving data quality (Klein et al. 2005).

To summarize, quality control included the call rate, the number of genotyped clusters, and consistency with the Hardy–Weinberg equilibrium test (P < 0.05). After the evaluation of quality control, the success rate of allele-identification was over 99%, and there was perfect agreement between the results of genotyping and direct sequencing, as previously described in our laboratory (Hamada et al. 2005; Kato et al. 2006).

Design of the extensive association study and statistical analyses

To bring down the cost and technical burden linked to genotyping, and to further reduce the type 1 error rate (false-positive) without losing any significant quality of data, we designed a two-stage association analysis strategy (Fig. 1). This approach is highly recommended to minimize the chance of false-positive association results (Hirschhorn and Daly 2005).

In the first association test samples, all SNPs were genotyped, and SNPs exhibiting the association with type 2 diabetes (P < 0.05) were further examined for replication in independent second test samples (Fig. 1 and Table 3). For each association analysis, the results of SNPs genotyping were calculated with four types of chi-square tests (allele frequency, genotype frequency, dominant model, and recessive model) with 2×2 , or 2×3 contingency tables. All analyses were two-tailed. As the cases were older than the controls in this study, a logistic regression model was used to adjust for age. However, this adjustment did not change the



Fig. 1 Scheme of the quality control and two-stage association study. Sample size and the number of SNPs are indicated. Full sets of SNPs are genotyped with a relatively small population (first association test). Only SNPs with P values < 0.05 for association with type 2 diabetes advanced to the next stage with a larger population for the purpose of replication (second association test)

significance level or patterns of association. Analysis was carried out using the SPSS system, release 12.0 J for Windows (SPSS Japan Inc., Tokyo, Japan).

Hardy–Weinberg equilibrium of alleles at individual loci was calculated with genotype frequency obtained by simple gene counting, and was evaluated by the chi-square test to compare observed and expected values. SNPs with a Hardy–Weinberg equilibrium test result of P < 0.05 in controls and cases were removed from the analysis (Klein et al. 2005).

To evaluate the type 1 error rate (false-positive) generated by testing a relatively large number of SNPs, standard Bonferroni's correction was used (McIntyre et al. 2000). In addition, we used the false-discovery rate (FDR) approach of Benjamini (Benjamini and Hochberg 1995) and Storey (Storey 2002), as implemented with the FDR control program in the R library. The FDR approach estimates the expected proportion of false positives among significant tests through q values. To obtain the q value for each marker, we used the QVALUE software (Storey and Tibshirani 2003). As a threshold, we used a value under 0.1 for SNPs associated with disease on the basis of a recent report (Shiffman et al. 2005).

As we evaluated numerous SNPs, it was necessary to process an enormous amount of genotyping data correctly. For this purpose, a high-speed resolution program, FGDS (Fujitsu Gene Discovery System; now available as ver 2.0, Fujitsu Ltd, Tokyo, Japan), was newly developed to efficiently process genotyping data in our laboratory. All algorithms using this system could modify the available programs in the public databases, and were strictly verified (Hamada et al. 2005; Kato et al. 2006).

Association study for Hepatocyte Nuclear Factor 4α (HNF4 α) gene variants

HNF4 α was considered as a putative susceptibility gene with type 2 diabetes, because several studies have observed suggestive linkage peaks around the HNF4 α

Table 3 The distribution of P values in 1,044 SNPs markers with the first association test

P value	Number	of SNPs								
	Allele n	nodel	Genoty	pe model	Domina	int model	Recessi	ve model	Overall	
$1 \ge P \ge 0.9$	115	11.0%	105	10.1%	104	10.0%	110	10.5%	10	1.0%
$0.9 > P \ge 0.8$	86	8.2%	89	8.5%	103	9.9%	93	8.9%	33	3.2%
$0.8 > P \ge 0.7$	99	9.5%	86	8.2%	103	9.9%	90	8.6%	44	4.2%
$0.7 > P \ge 0.6$	105	10.1%	81	7.8%	97	9.3%	87	8.3%	74	7.1%
$0.6 > P \ge 0.5$	79	7.6%	91	8.7%	112	10.7%	93	8.9%	79	7.6%
$0.5 > P \ge 0.4$	93	8.9%	114	10.9%	89	8.5%	99	9.5%	86	8.2%
$0.4 > P \ge 0.3$	103	9.9%	108	10.3%	95	9.1%	102	9.8%	119	11.4%
$0.3 > P \ge 0.2$	113	10.8%	117	11.2%	112	10.7%	103	9.9%	144	13.8%
$0.2 > P \ge 0.1$	126	12.1%	118	11.3%	111	10.6%	131	12.5%	201	19.3%
$0.1 > P \ge 0.05$	64	6.1%	48	4.6%	62	5.9%	71	6.8%	112	10.7%
$0.05 > P^{-}$	61	5.8%	87	8.3%	56	5.4%	65	6.2%	142	13.6%
Total	1,044		1,044		1,044		1,044		1,044	

Data are presented as the number of SNPs. P values are calculated by four chi-square tests, allele, genotype, dominant and recessive models. 142 SNPs revealed a P value of < 0.05 in at least one of the four type chi-square tests

gene on chromosome 20q in multiple populations (Love Gregory et al. 2004; Silander et al. 2004).

Seventeen SNPs markers were arranged around the HNF4 α locus including the pancreatic beta cell P2 promoter on the basis of two recent publications containing significant SNPs associated with disease (Winckler et al. 2005; Bagwell et al. 2005), and were examined with 1,818 samples (both first and second test samples). In this evaluation, mis-sense variant Thr130Ile (rs1800961) was included (Zhu et al. 2003). The average spacing between SNPs markers was approximately one per 5.9 kb across this locus. After the pattern and extent of linkage disequilibrium (LD) were examined, a haplotype-based association test was also analyzed.

Statistical power and sample size calculation for association study

To ensure adequate statistical results with an association study, power calculation was simulated with the PS power and sample-size program. Power was the probability correctly rejecting the null hypothesis that the odds ratio was equal between cases and controls. Power in the association study was dependent on several factors: allele frequency, the type 1 error (falsepositive) rate, the odds ratio, and the sample size.

Our simulation showed that a sample size of 900 cases and 900 controls would reach 60–80% power to detect an odds ratio as small as 1.2–1.5, when predisposing an allele frequency with a type 1 error rate of 0.05 (Supplementary Fig. 1). In this simulation, the power was low for uncommon rare variants. In general, rare alleles require larger samples to attain the same power, as compared to common frequent alleles. When adjusting the type 1 error rate to less than 0.01 to compensate for multiple testing, much larger samples were required than reported here. Recent reports indicated that the odds ratio with type 2 diabetes was likely within 1.2–1.5 (O'Rahilly et al. 2005; Barroso et al. 2003).

Re-sequencing and discovery of SNPs around a landmark SNP

After a landmark SNP was selected as a putative disease-susceptibility variant within the target region, additional SNPs were arranged by searching the public databases. In addition, to discover unidentified SNPs not listed in databases, we screened SNPs by re-sequencing the genes around a landmark SNP in 24 individuals with type 2 diabetes.

On the basis of the genomic sequence in the public databases, primers were designed using Primer 3 program covering all exons and flanking intronic sequences. In addition, we designed primers to amplify 10 kb upstream of the first exon and 10 kb downstream of the last exon. Details of the primer sequences are available upon request. With few exceptions, PCR primers were designed to amplify an 800-900 bp fragment with a more than 100 bp overlap between amplicons. Amplification primers were also used for sequencing. All PCR products were prepared for sequence analysis by treatment with ExoSAP-IT (Amersham Biosciences, Piscataway, NJ) at 37°C for 15 min, followed by incubation at 80°C for 15 min to deactivate the enzyme. PCR products were sequenced using an ABI BigDye terminator cycle sequencing kit, ver 1.1 (Applied Biosystems) in both directions according to standard protocols. Analysis was performed on an ABI 3100 or ABI 3730xl automated sequencer (both from Applied Biosystems).

Pairwise linkage disequilibrium definition

LD was defined as the statistical association between alleles at two or more sites (Wall and Pritchard 2003). Among them, |D'| and r^2 were used as the standard approach in this study. These values were calculated with FGDS and SNPAlyze ver 3.2.2 Pro software (DYNACOM, Yokohama, Japan).

- 1. The classical statistical |D'| threshold approach (Lewontin 1964): A LD was defined whenever all pairwise |D'| between three or more successive markers exceeded 0.9. Pairwise |D'| coefficients were calculated using simple sliding window assessment.
- 2. The value of r^2 definition (Hill and Robertson 1968): r^2 was the square of the correlation coefficient between markers and ranged from 0 to 1. The maximum possible value depended on the minor allele frequency of two markers. Thus, the value of r^2 was 1 when two SNPs arose on the same branch of the genealogy and remained undisrupted by recombination. However, the value was less than 1 when SNPs arose on different branches.

Computational haplotype inference and analysis

In this study, we used two different methods for haplotype inference based on the measured genotyped data, each of which provided different and complementary approaches.

- 1. By the Bayesian statistical method, haplotypes were implemented from genotype data with PHASE ver 2.1 program. This method regarded the unknown haplotypes as unobserved random quantities and aimed to evaluate their conditional distribution in light of the genotype data. We used the default parameter values (100 iterations, a thinning interval of 1, and a burn-in value of 100) specified in the program to estimate haplotype frequency (Stephens et al. 2001).
- 2. As an alternative method, haplotype frequencies for multiple loci were estimated with the expectation-maximization (EM) algorithm in SNPAlyze ver 3.2.2 Pro software (DYNACOM). This procedure was an interactive method obtaining maximum-likelihood, and haplotype frequencies were estimated from multi-locus genotype data when the gametic phase was unknown (Ohmori et al. 2004).

In addition, the permutation method was used to test the deviation of allelic frequencies of haplotypes. Distribution of a test statistic was estimated by evaluating the statistics for a random sampling of 10,000 iterated permutations with a fixed total number of both cases and controls, which was incorporated in SNPAlyze ver 3.2.2. The measurement of the *P* value was estimated by the proportion of permutations for which the permutated data test statistic ($P_{\text{permutated}}$) was greater than the initially observed test statistic (P_{observed}), so permutation $P = P(P_{\text{observed}} > P_{\text{permutated}})$ (Jinnai et al. 2004).

Uniform Resource Locators (URLs) of computer programs used in this study

PS power and sample-size program: http://www. biostat.mc.vanderbilt.edu/twiki/bin/view/Main/Power SampleSize.

Primer 3: http://www.frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi.

QVALUE (FDR program): http://www.faculty.washington.edu/~jstorey/qvalue/index.html.

PHASE ver 2.1: http://www.stat.washington.edu/ stephens/software.html.

SNPAlyze ver 3.2.2: http://www.dynacom.co.jp/e/products/package/snpalyze/index.html.

Pharma SNP consortium (PSC): http:// www.jpma.or.jp/psc/frame-e.html.

URLs of public databases used in this study

HapMap project: http://www.hapmap.org/.

dbSNP (now Build 125 is available): http:// www.ncbi.nlm.nih.gov/SNP/.

NCBI Build 35 human genomes: http:// www.ncbi.nlm.nih.gov/Genomes/.

PubMed database: http://www.ncbi.nlm.nih.gov/en-trez/.

Results

Association test for HNF4 α gene variants

Single-marker association test

We evaluated 17 SNPs covering a 100-kb region spanning distal to the pancreatic beta cell P2 promoter through to the end of the coding sequence at an average of one per 5.9 kb. All SNPs were located in the non-coding locus except rs1800961 (exon 4), resulting in a Thr130Ile amino acid change (Zhu et al. 2003). In an association study, 893 control and 925 case samples were used. The results of the association study and the Hardy-Weinberg equilibrium test are summarized in Table 4A. In controls, five SNPs were not confirmed in the Hardy-Weinberg equilibrium test. Three consecutive SNPs (rs2273618, rs6073435, and rs6031601) from intron 7 to 9 were found to be associated with disease (P = 0.0458, 0.0426, and 0.0421; allele model). However, the magnitude of P values was extremely weak and spurious when evaluated by multiple testing (corrected for 17 tests). In our study, the variant with rs6031601 was mostly associated with disease, but was not observed in Caucasians (Winckler et al. 2005; Bagwell et al. 2005; Silander et al. 2004; Love Gregory et al. 2004).

In the FUSION (The Finland-United States Investigation of Non-insulin-dependent diabetes mellitus genetics) study and Ashkenazi Jews, associations were observed with variants in the P2 promoter region. However, a recent study failed to replicate the association with these variants in more than 7,000 samples (Winckler et al. 2005). We were also unable to confirm the association of four SNPs in the P2 promoter region (rs4810424, rs1884613, rs1884614, and rs2144908). In this region, there were quite different allele frequencies among our samples (including HapMap JPT samples) and HapMap CEU samples (Supplementary Table 2A).

Recently, a rare loss-of-function SNPs (rs1800961) in exon 4 was associated with type 2 diabetes in Japanese (Zhu et al. 2003). However, our results failed to confirm the association, although we examined more than twice as many samples.

Cyto- No. bands	SNP ID	dbSNP ID	Genes	Location	Position	SNP allele	Minor frequenc	allele <i>P</i> value y versus	e (first) (358 cont	367 case rols)	s	P value (versus 53	second) 5 contro	(558 cases ds)	P value versus 8	(overall 393 contr) (925 ca ols)	ses	Odds ratio	s 95% CI	P value
						Allele 1/2	Control	Case Allele	Geno- type	Domi- nant	Reces- sive	Allele	Geno- I type r	Jomi- Recessive lant	Allele	Geno- type	Domi- nant	Reces- sive			Hardy Weinbo equilibi test
(A) 17SNP	s around th	e HNF4¤ lc	cus																		
q13.12 1	SNP2258	rs4810424	HNF4α	P2promotor	42,408,437	G/C	0.442	0.462 0.08	0.22	0.09	0.29	0.92	0.77	0.77 0.62	0.24	0.52	0.42	0.29	NS	SN	0.02
2		rs1884613	HNF4α	P2promotor	42,413,829	C/G	0.474	0.477 0.27	0.51	0.28	0.46	0.47	0.57	0.30 0.92	0.89	0.89	0.89	0.69	NS	SN	0.93
33		rs1884614	HNF4α	P2promotor	42,413,933	CT	0.476	0.482 0.25	0.46	0.23	0.48	0.68	0.59	0.39 0.82	0.68	0.81	0.96	0.53	NS	NS	1.00
4		rs2144908	HNF4α	P2promotor	42,419,131	G/A	0.477	0.487 0.25	0.42	0.55	0.19	0.86	0.49	0.57 0.43	0.55	0.72	0.42	0.86	SS	NS	0.97
5		rs6073418	HNF4α	I	42,434,004	CT	0.220	0.210 0.29	0.11	0.11	0.38	0.89	0.96	0.97 0.78	0.46	0.50	0.31	0.80	NS	NS	0.64
9	SNP1383	rs2071197	HNF4α	Intron 1	42,463,849	G/A	0.456	0.446 0.19	0.30	0.62	0.12	0.74	0.26	0.23 0.57	0.56	0.17	0.54	0.16	SS	NS	0.28
7	SNP1384	rs736824	HNF4α	Intron 1	42,468,074	CT	0.259	0.255 0.23	0.20	0.11	0.82	0.17	0.42	0.22 0.37	0.76	0.66	0.94	0.40	NS	NS	0.08
8		rs1885088	HNF4α	Intron 3	42,472,454	G/A	0.007	0.003 0.97	0.97	0.97	I	0.07	0.07	0.07 -	0.14	0.14	0.14	I	NS	NS	0.02
6		rs1885089	HNF4α	Intron 3	42,472,677	G/A	0.007	0.003 0.68	0.68	I	0.68	0.07	0.07	- 0.07	0.09	0.09	I	0.09	NS	NS	0.04
10		rs1800961	HNF4α	Exon 4	42,475,778	CT	0.010	0.016 0.21	0.21	I	0.21	0.19	0.19	- 0.19	0.07	0.07	I	0.07	SS	NS	0.14
11	SNP1385	rs3212198	HNF4α	Intron 5	42,477,776	СЛ	0.331	0.352 0.29	0.29	0.12	0.46	0.61	0.72	0.46 0.72	0.18	0.30	0.12	0.68	NS	NS	0.28
12	SNP2259	rs1028583	HNF4α	Intron 7	42,484,175	T/G	0.306	0.328 0.26	0.26	0.73	0.10	0.47	0.26	0.59 0.26	0.15	0.08	0.84	0.04281	NS	NS	0.04
13		rs1028584	HNF4α	Intron 7	42,484,395	A/C	0.336	0.358 0.54	0.54	0.27	0.56	0.37	0.41	0.21 0.41	0.17	0.24	0.10	0.74	NS	NS	0.44
14		rs2273618	HNF4α	Intron 7	42,485,984	C/T	0.377	0.410 0.34	0.34	0.50	0.14	0.15	0.31	0.45 0.31	0.04588	0.11	0.31	0.03649	1.15	1.002-1.	309 0.45
15	SNP1386	rs6073435	HNF4α	Intron 8	42,487,002	A/T	0.343	0.375 0.31	0.31	0.13	0.55	0.15	0.29	0.12 0.29	0.04266	0.09	0.02984	0.38	1.15	1.005-1.	318 0.13
16	SNP2260	rs6031601	HNF4α	Intron 9	42,491,234	C/A	0.351	0.383 0.31	0.31	0.51	0.13	0.15	0.34	0.20 0.34	0.04211	0.13	0.16	0.07	1.15	1.005-1	319 0.57
17		rs911358	HNF4α	I	42,508,442	T/A	0.117	0.117 0.15	0.15	0.05	0.85	0.64	0.77	0.48 0.77	0.98	0.86	0.62	0.92	NS	NS	0.93
(B) 20 SNF	s around a	landmark S	NP locus																		
q11.23 1	SNP2076	rs2425229	DLGAP4	Intron	34,440,834	A/G	0.355	0.370 0.54	0.38	0.38	0.95	I	I	I I	I	I	I	I	NS	NS	0.76
2	SNP113	rs2425236	DLGAP4	Intron	34,445,973	G/C	0.357	0.375 0.48	0.26	0.26	0.13	I	I	I	I	I	I	I	NS	NS	0.52
33	SNP1140	rs2425242	DLGAP4	Intron	34,452,333	T/C	0.359	0.372 0.61	0.29	0.29	0.17	I	I	I	I	I	I	I	SN	NS	0.46
4	SNP1143	rs6124857	DLGAP4	Intron	34,484,621	A/G	0.419	0.450 0.24	0.43	0.43	0.20	I	I	I	I	I	I	I	SS	NS	0.96
5	SNP1144	rs1275396	DLGAP4	Intron	34,501,432	G/A	0.356	0.389 0.11	0.23	0.23	0.09	0.21	0.43	0.28 0.30	0.04339	0.12	0.19	0.06	1.15	1.004-1.	316 0.91
9		rs1275404	DLGAP4	Intron	34,530,533	A/G	0.245	0.259 0.14	0.28	0.28	0.20	0.99	0.66	0.49 0.72	0.33	0.54	0.84	0.27	SS	NS	0.72
7		rs1275398	DLGAP4	Intron	34,540,383	G/A	0.060	0.055 0.38	0.48	0.48	0.32	0.13	0.31	$0.14 \ 0.54$	0.55	0.83	0.54	0.97	SS	NS	0.66
8	SNP1145	rs220079	DLGAP4	3'-UTR	34,589,556	G/A	0.425	0.472 0.08	0.18	0.18	0.23	0.02974	0.09	0.13 0.04	0.00461	0.01812	0.02388	0.01797	1.21	1.060 - 1	380 1.00
6	SNP1146	rs220076	6TAM	Intron	34,605,971	C/A	0.432	0.483 0.04055	0.07	0.07	0.30	0.02626	0.06 0	017 0.02	0.00231	0.01010	0.01157	0.01507	1.23	1.077 - 1	399 0.93
10		rs694379	I		34,612,910	CT	0.428	0.474 0.06	0.15	0.15	0.18	0.04714	012	0.20 0.05	0.00563	0.02091	0.03319	0.01643	1.20	1.056-1.	373 0.94
11		rs6071089	I		34,617,113	CT	0.497	0.475 0.10	0.20	0.20	0.35	0.43	0.22	0.13 0.79	0.09	0.07	0.02	0.68	NS	NS	0.13
12	SNP1147	rs85440	TGIF2	Intron	34,648,765	G/A	0.407	0.432 0.11	0.10	0.10	0.03463	0.53	0.14	0.09 0.67	0.12	0.25	0.11	0.30	NS	NS	0.18
13	SNP1148	rs555394	C20orf24	Intron	34,670,739	G/A	0.233	0.259 0.26	0.48	0.48	0.66	I	I	I	I	I	I	I	NS	NS	0.40
14	SNP2081	rs693361	SLA2	Intron	34,684,087	A/G	0.227	0.255 0.21	0.44	0.44	0.25	I	I	I	I	I	I	I	NS	NS	0.42
15	SNP2082	rs221311	NDRG3	Intron	34,719,874	СŢ	0.236	0.271 0.13	0.13	0.13	0.94	I			I	I	I	I	NS	NS	0.52
16	SNP2083	rs221314	NDRG3	Intron	34,732,082	A/G	0.256	0.247 0.69	0.85	0.85	0.60	I	I	1	I	I	I	I	NS	NS	0.70
17	SNP1149	rs221307	NDRG3	Intron	34,748,455	A/G	0.309	0.309 0.99	0.93	0.93	0.78	I	I	I I	I	I	I	I	NS	NS	0.96
18	SNP1150	rs6129459	NDRG3	Intron	34,748,581	T/C	0.312	0.306 0.81	0.95	0.95	1.00	I	I	1	I	I	I	I	NS	NS	0.87

P value	Hardy Weinberg equilibrium test	0.19 0.29	around a sts, allele, e decimal ss < 0.05
95% CI		NS NS	locus are tes ith fiv
Odds ratio		NS NS	r the ni-squi ace wi
ases	Reces- sive	1 1	alysis fo type ch n boldfi mples.
l) (925 c rols)	Domi- nant	1 1	he an: h four 5 are i trol sa
s (overal 893 cont	Geno- type	1 1	ed to t ed wit < 0.0 in con
P value versus {	Allele	1 1	e adde alculat alues 1 only
ses	Recessive	1 1	NPs wer s were c ninal P v are shown
(558 cas ls)	Jomi- nant		r dbS value ne non test a
second) 5 contro	Jeno- J ype 1		vith fou base. <i>H</i> ults. Th librium
⁹ value (rersus 53	Allele (sults w e data pe resu g equi
	Reces- 1	0.55 - 0.61 -	(B) Re sequenc genoty Veinber
867 cases ols)	Domi- nant	0.53 0.55	l locus. 2nome a 7ailable Iardy-V
(first) (3 58 contr	Geno- type	0.53 0.55	HNF4a man ge the av with F
P value versus 3	Allele	0.79 0.72	r the] 35 hu d with values
allele y	Case	0.331 0.313	ysis fc Build lculate nt'. P
Minor frequenc	Control	0.338 0.322	he anal e NCBI were ca
SNP allele	Allele 1/2	G/A C/T	ed to the on the tests v to to the tests v
Position		34,786,132 34,794,053	were add ere based Thi-square terval. M
Location		Intron Intron	dbSNPs sitions w nodels. C dence ir
Genes		NDRG3 NDRG3	with 10 c SNPs por cessive m
dbSNP ID		rs6072018 rs2425266	notyping ' mes and ! nt and rec lenotes 95
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/to- No. nds		19 20	A) Resul ndmark motype, shumns. 9
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Haplotype-based association test

Nevertheless, it is possible that haplotypes might be associated with increased or decreased disease risks, although individual SNPs are weakly associated with disease. Similar to other populations (Finnish and Ashkenazi Jews), four SNPs in the P2 promoter region showed high LD values (|D'| > 0.99 and $r^2 > 0.89$) (Table 5A). With the interpretation of r^2 , the coding region was divided into the short LD blocks. Based on the interpretation of r^2 , there were two different haplotype blocks around the HNF4 α gene, such as one in the P2 promoter region and another in the coding region.

We analyzed these two haplotypes to certify the haplotype-based association test. No haplotypes in the P2 promoter region were associated with disease (data not shown), similar to the results in the single-marker association test. When one haplotype with three SNPs encompassing intron 7–9 was examined, it was weakly associated with controls and cases (Table 6A). However, this association could be spurious because the magnitude of the *P* value failed to satisfy Bonferroni's correction (corrected for 17 plus 8 tests).

Target region on chromosome 20q and SNPs markers

To widen the observations, we enlarged the analysis region on the basis of previous linkage reports. The putative disease susceptibility locus on chromosome 20q was certainly mapped to the 50.81–75.01 cM region, corresponding to a 19.31-Mb interval. Within the genomic region, 291 genes were found in the National Center for Biotechnology Information (NCBI) Build 35 human genome assembly (Table 2).

To establish a useful and dense SNPs marker set, we first searched the public and Celera databases and developed TaqMan assays for 631 SNPs markers. In addition, to collect enough SNPs markers for fine analysis, custom SNPs markers (Custom TaqMan SNP Genotyping Assays) were especially designed for the other 516 positions. As a result, 1,147 SNPs marker probes were assayed, covering the target region with an average of one per 10.8-kb interval. Of the 1,147 SNPs genotyped in the target region, 103 SNPs were excluded by quality control criteria (see Materials and methods), mainly because of deviation in the Hardy-Weinberg equilibrium test (Figs. 1, 2). Of 291 genes within the target region, 219 genes (75.3%) were arranged with at least one SNPs marker. All information regarding the 1,147 SNPs markers is listed in Supplementary Table 3.

No.	SNP ID	dbSNP ID	Gene	Location	Position		1	2	3	- 4	5	6	7	8	9	10	11	12	13	14	15	16	17	_		\mathbf{D}	
							rs4810424	rs1884613	rs1884614	rs2144908	rs6073418	rs2071197	rs736824	rs1885088	rs1885089	rs1800961	rs3212198	rs1028583	8 rs1028584	rs2273618	rs6073430	5 rs6031601	rs911358		-		
1	SNP2258	rs4810424	$HNF4\alpha$	P2 promotor	42,408,437			0.991	1	1	0.590	0.077	0.461	0.427	0.445	0.576	0.010	0.013	0.035	0.021	0.018	0.002	0.148				0.95 < 1
2		rs1884613	$HNF4\alpha$	P2 promotor	42,413,829		0.893		1	1	0.594	0.112	0.505	0.366	0.372	0.568	0.000	0.021	0.026	0.031	0.036	0.002	0.209				0.90 < 1
3		rs1884614	$HNF4\alpha$	P2 promotor	42,413,933		0.902	0.992		1	0.599	0.120	0.511	0.356	0.360	0.581	0.010	0.012	0.020	0.021	0.026	0.008	0.196				0.85 <
4		rs2144908	$HNF4\alpha$	P2 promotor	42,419,131		0.890	0.978	0.986		0.594	0.125	0.508	0.345	0.345	0.596	0.018	0.004	0.011	0.018	0.022	0.013	0.204				
5		rs6073418	$HNF4\alpha$	•	42,434,004		0.073	0.081	0.083	0.083		0.225	0.188	0.358	0.677	0.017	0.118	0.189	0.150	0.118	0.161	0.003	0.553				
6	SNP1383	rs2071197	$HNF4\alpha$	Intron 1	42,463,849		0.006	0.011	0.013	0.014	0.011		1	0.99986	0.99995	0.99998	0.573	0.492	0.547	0.465	0.414	0.446	0.120				
7	SNP1384	rs736824	$HNF4\alpha$	Intron 1	42,468,074		0.062	0.082	0.085	0.086	0.003	0.295		0.99999	1	0.998	0.048	0.064	0.051	0.053	0.246	0.105	0.065				
8		rs1885088	$HNF4\alpha$	Intron 3	42,472,454		0.001	0.001	0.001	0.001	0.00013	0.004	0.013		1	1	0.99999	1	0.99999	0.99998	1	0.99999	1				
9		rs1885089	$HNF4\alpha$	Intron 3	42,472,677	r^2	0.001	0.001	0.001	0.001	0.001	0.004	0.014	1	-	1	0.99999	1	0.99999	0.99999	0.99999	0.99999	1	I D'			
10		rs1800961	$HNF4\alpha$	Exon 4	42,475,778		0.003	0.003	0.003	0.004	0.00001	0.011	0.004	0.00004	0.00005		0.99999	1	0.99999	0.99999	1	0.99999	0.014				
11	SNP1385	rs3212198	$HNF4\alpha$	Intron 5	42,477,776		0.00006	0	0.00005	0.00016	0.007	0.144	0.002	0.008	0.010	0.020		0.892	0.941	0.934	0.779	0.882	0.082				
12	SNP2259	rs1028583	$HNF4\alpha$	Intron 7	42,484,175		0.00009	0.00021	0.00006	0.00001	0.018	0.098	0.001	0.009	0.011	0.023	0.726		0.997	1	0.978	0.841	0.244				
13		rs1028584	$HNF4\alpha$	Intron 7	42,484,395		0.001	0.00038	0.00020	0.00006	0.010	0.136	0.002	0.008	0.009	0.020	0.847	0.871		1	0.851	0.860	0.073			r^2	
14		rs2273618	HNF4g	Intron 7	42,485,984		0.00034	0.001	0.00030	0.00021	0.005	0.123	0.001	0.006	0.007	0.016	0.675	0.709	0.803		1	0.990	0.242			-	
15	SNP1386	rs6073435	HNF4g	Intron 8	42,487,002		0.00021	0.001	0.00040	0.00027	0.011	0.084	0.011	0.008	0.009	0.019	0.543	0.783	0.668	0.867		0.863	0.038				0.95 < 1
16	SNP2260	rs6031601	HNF4α	Intron 9	42,491,234		0	0	0.00004	0.00010	0	0.101	0.002	0.007	0.008	0.018	0.679	0.565	0.667	0.869	0.729		0.247				0.90 < 1
17		rs911358	$HNF4\alpha$		42,508,442		0.004	0.007	0.006	0.006	0.011	0.002	0.00021	0.001	0.001	0.00001	0.002	0.004	0.002	0.013	0.00039	0.016					0.85 < 1
				На	aplotype bloc	ks I D	1																				
					apiotype bloc																						
(B)	Pairwis	se LD of 2	0 SNPs a	around a la	ndmark SNF	? locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	
(B)	Pairwis SNP ID	dbSNP ID	Genes	Location	Position	? locus	1 rs2425229	2 rs2425236	3 rs2425242	4 rs6124857	5 rs1275396	6 rs1275404	7 rs1275398	8 rs220079	9 rs220076	10 rs694379	11 rs6071089	12 rs85440	13 rs555394	14 rs693361	15 rs221311	16 rs221314	17 rs221307	18 rs6129459	19 rs6072018	20 rs2425266	
(B) No.	Pairwis SNP ID SNP2076	dbSNP ID	Genes	Location	Position 34,440,834	? locus	1 rs2425229	2 rs2425236 1	3 rs2425242 0.997	4 rs6124857 0.298	5 rs1275396 0.096	6 rs1275404 0.006	7 rs1275398 0.541	8 rs220079 0.100	9 rs220076 0.092	10 rs694379 0.086	11 rs6071089 0.051	12 rs85440 0.092	13 rs555394 0.115	14 rs693361 0.134	15 rs221311 0.034	16 rs221314 0.075	17 rs221307 0.025	18 rs6129459 0.060	19 rs6072018 0.031	20 rs2425266 0.109	
(B) No.	Pairwis SNP ID SNP2076 SNP1139	se LD of 2 dbSNP ID rs2425229 rs2425236	Genes DLGAP4 DLGAP4	Location Intron Intron	Position 34,440,834 34,445,973	? locus	1 rs2425229 0.986	2 rs2425236	3 rs2425242 0.997 1	4 rs6124857 0.298 0.298	5 rs1275396 0.096 0.104	6 rs1275404 0.006 0.013	7 rs1275398 0.541 0.554	8 rs220079 0.100 0.109	9 rs220076 0.092 0.101	10 rs694379 0.086 0.098	11 rs6071089 0.051 0.069	12 rs85440 0.092 0.103	13 rs555394 0.115 0.113	14 rs693361 0.134 0.139	15 rs221311 0.034 0.041	16 rs221314 0.075 0.079	17 rs221307 0.025 0.010	18 rs6129459 0.060 0.042	19 rs6072018 0.031 0.015	20 rs2425266 0.109 0.095	-
No.	Pairwis SNP ID SNP2076 SNP1139 SNP1140	dbSNP ID rs2425229 rs2425236 rs2425242	Genes DLGAP4 DLGAP4 DLGAP4 DLGAP4	Location Intron Intron Intron	Position 34,440,834 34,445,973 34,452,333	? locus	1 rs2425229 0.986 0.983	2 rs2425236 1 0.997	3 rs2425242 0.997	4 rs6124857 0.298 0.298 0.301	5 rs1275396 0.096 0.104 0.103	6 rs1275404 0.006 0.013 0.013	7 rs1275398 0.541 0.554 0.556	8 rs220079 0.100 0.109 0.110	9 rs220076 0.092 0.101 0.103	10 rs694379 0.086 0.098 0.097	11 rs6071089 0.051 0.069 0.066	12 rs85440 0.092 0.103 0.100	13 rs555394 0.115 0.113 0.118	14 rs693361 0.134 0.139 0.145	15 rs221311 0.034 0.041 0.042	16 rs221314 0.075 0.079 0.073	17 rs221307 0.025 0.010 0.018	18 rs6129459 0.060 0.042 0.053	19 rs6072018 0.031 0.015 0.028	20 rs2425266 0.109 0.095 0.102	
(B) No.	Pairwis SNP ID SNP2076 SNP1139 SNP1140 SNP1143	te LD of 2 dbSNP ID rs2425229 rs2425236 rs2425242 rs6124857	Genes DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4	Location Intron Intron Intron Intron Intron	Position 34,440,834 34,445,973 34,452,333 34,452,333	Plocus	1 rs2425229 0.986 0.983 0.067	2 rs2425236 1 0.997 0.068	3 rs2425242 0.997 1 0.069	4 rs6124857 0.298 0.298 0.301	5 rs1275396 0.096 0.104 0.103 0.524	6 rs1275404 0.006 0.013 0.013 0.541	7 rs1275398 0.541 0.554 0.556 0.555	8 rs220079 0.100 0.109 0.110 0.404	9 rs220076 0.092 0.101 0.103 0.436	10 ns694379 0.086 0.098 0.097 0.407	11 rs6071089 0.051 0.069 0.066 0.427	12 rs85440 0.092 0.103 0.100 0.345	13 rs555394 0.115 0.113 0.118 0.596	14 rs693361 0.134 0.139 0.145 0.589	15 rs221311 0.034 0.041 0.042 0.386	16 rs221314 0.075 0.079 0.073 0.158	17 rs221307 0.025 0.010 0.018 0.316	18 rs6129459 0.060 0.042 0.053 0.311	19 rs6072018 0.031 0.015 0.028 0.290	20 rs2425266 0.109 0.095 0.102 0.259	
(B) No.	Pairwis SNP ID SNP2076 SNP1139 SNP1140 SNP1143 SNP1144	se LD of 2 dbSNP ID rs2425229 rs2425236 rs2425242 rs6124857 rs1275396	Genes DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4	Intron Intron Intron Intron Intron Intron Intron	endmark SNF Position 34,440,834 34,445,973 34,452,333 34,452,333 34,484,621 34,501,432	Plocus	1 182425229 0.986 0.983 0.067 0.009	2 rs2425236 1 0.997 0.068 0.010	3 rs2425242 0.997 1 0.069 0.010	4 rs6124857 0.298 0.301 0.216	5 rs1275396 0.096 0.104 0.103 0.524	6 rs1275404 0.006 0.013 0.013 0.541 0.985	7 rs1275398 0.541 0.554 0.556 0.555 0.975	8 rs220079 0.100 0.109 0.110 0.404 0.889	9 rs220076 0.092 0.101 0.103 0.436 0.868	10 rs694379 0.086 0.098 0.097 0.407 0.881	11 rs6071089 0.051 0.069 0.066 0.427 0.946	12 rs85440 0.092 0.103 0.100 0.345 0.640	13 rs555394 0.115 0.113 0.118 0.596 0.850	14 rs693361 0.134 0.139 0.145 0.589 0.821	15 rs221311 0.034 0.041 0.042 0.386 0.663	16 rs221314 0.075 0.079 0.073 0.158 0.175	17 rs221307 0.025 0.010 0.018 0.316 0.462	18 rs6129459 0.060 0.042 0.053 0.311 0.452	19 rs6072018 0.031 0.015 0.028 0.290 0.377	20 rs2425266 0.109 0.095 0.102 0.259 0.376	-
(B) No. 1 2 3 4 5 6	Pairwis SNP ID SNP2076 SNP1139 SNP1140 SNP1143 SNP1144	dbSNP ID rs2425229 rs2425236 rs2425236 rs2425242 rs6124857 rs1275396 rs1275404	Genes DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4	Intron Intron Intron Intron Intron Intron Intron Intron	Position 34,440,834 34,445,973 34,452,333 34,452,333 34,484,621 34,501,432 34,500,533	? locus	1 rs2425229 0.986 0.983 0.067 0.009 0.00002	2 rs2425236 1 0.997 0.068 0.010 0.0001	3 rs2425242 0.997 1 0.069 0.010 0.0001	4 rs6124857 0.298 0.301 0.216 0.128	5 rs1275396 0.096 0.104 0.103 0.524 0.535	6 rs1275404 0.006 0.013 0.013 0.541 0.985	7 rs1275398 0.554 0.555 0.555 0.975 0.982	8 rs220079 0.100 0.109 0.110 0.404 0.889 0.852	9 rs220076 0.092 0.101 0.103 0.436 0.868 0.823	10 rs694379 0.086 0.098 0.097 0.407 0.881 0.832	11 rs6071089 0.051 0.069 0.066 0.427 0.946 0.924	12 rs85440 0.092 0.103 0.100 0.345 0.640 0.838	13 rs555394 0.115 0.113 0.118 0.596 0.850 0.840	14 rs693361 0.134 0.139 0.145 0.589 0.821 0.822	15 rs221311 0.034 0.041 0.042 0.386 0.663 0.598	16 rs221314 0.075 0.079 0.073 0.158 0.175 0.703	17 rs221307 0.025 0.010 0.018 0.316 0.462 0.593	18 rs6129459 0.060 0.042 0.053 0.311 0.452 0.591	19 rs6072018 0.031 0.015 0.028 0.290 0.377 0.579	20 rs2425266 0.109 0.095 0.102 0.259 0.376 0.592	
(B) No. 1 2 3 4 5 6 7	Pairwis SNP ID SNP2076 SNP1139 SNP1140 SNP1143 SNP1144	dbSNP ID rs2425229 rs2425236 rs2425236 rs2425242 rs6124857 rs1275396 rs1275398	Genes DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4	Location Intron Intron Intron Intron Intron Intron Intron	Position 34,440,834 34,445,973 34,445,2,333 34,484,621 34,501,432 34,501,432 34,500,533 34,540,383	? locus	1 rs2425229 0.986 0.983 0.067 0.009 0.00002 0.033	2 rs2425236 1 0.0997 0.068 0.010 0.0001 0.033	3 rs2425242 0.997 1 0.069 0.010 0.0001 0.033	4 rs6124857 0.298 0.301 0.216 0.128 0.025	5 1 rs1275396 0.096 0.104 0.103 0.524 0.535 0.100	6 rs1275404 0.006 0.013 0.013 0.013 0.013 0.541 0.985 0.183	7 10.554 0.555 0.555 0.975 0.982	8 rs220079 0.100 0.109 0.110 0.404 0.889 0.852 0.653	9 rs220076 0.092 0.101 0.103 0.436 0.868 0.823 0.619	10 rs694379 0.086 0.097 0.407 0.881 0.832 0.653	11 rs6071089 0.051 0.069 0.427 0.946 0.924 1	12 rs85440 0.092 0.103 0.100 0.345 0.640 0.838 0.635	13 rs555394 0.115 0.113 0.118 0.596 0.850 0.840 0.725	14 rs693361 0.134 0.139 0.145 0.589 0.821 0.822 0.712	15 rs221311 0.034 0.041 0.042 0.386 0.663 0.598 0.667	16 rs221314 0.075 0.079 0.073 0.158 0.175 0.703 0.99999	17 rs221307 0.025 0.010 0.018 0.316 0.462 0.593 0.894	18 rs6129459 0.060 0.042 0.053 0.311 0.452 0.591 0.889	19 rs6072018 0.031 0.015 0.028 0.290 0.377 0.579 0.885	20 rs2425266 0.109 0.095 0.102 0.259 0.376 0.592 0.892	
(B) No. 1 2 3 4 5 6 7 8	Pairwis SNP ID SNP2076 SNP1139 SNP1140 SNP1143 SNP1144 SNP1145	e LD of 2 dbSNP ID rs2425229 rs2425236 rs2425242 rs6124857 rs1275396 rs1275398 rs1275398 rs220079	Genes DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4	Location Intron Intron Intron Intron Intron Intron Intron 3-UTR	Pesition 34,440,834 34,440,834 34,445,973 34,445,2333 34,484,621 34,501,432 34,503,333 34,540,383 34,589,556	? locus	1 rs2425229 0.986 0.983 0.067 0.009 0.00002 0.0033 0.007	2 rs2425236 1 0.997 0.068 0.010 0.0001 0.033 0.008	3 rs2425242 0.997 1 0.069 0.010 0.0001 0.033 0.009	4 rs6124857 0.298 0.298 0.216 0.128 0.025 0.153	5 0.096 0.104 0.103 0.524 0.535 0.100 0.580	6 rs1275404 0.006 0.013 0.013 0.541 0.985 0.183 0.295	7 151275398 0.541 0.554 0.555 0.955 0.975 0.982 0.032	8 rs220079 0.100 0.109 0.110 0.404 0.889 0.852 0.653	9 rs220076 0.092 0.101 0.103 0.436 0.456 0.4	10 rs694379 0.086 0.098 0.097 0.407 0.881 0.832 0.653 0.973	11 rs6071089 0.051 0.069 0.066 0.427 0.946 0.924 1 0.928	12 rs85440 0.092 0.103 0.100 0.345 0.640 0.838 0.635 0.892	13 rs555394 0.115 0.113 0.118 0.596 0.850 0.840 0.725 0.955	14 rs693361 0.134 0.139 0.145 0.589 0.821 0.822 0.712 0.926	15 rs221311 0.034 0.041 0.042 0.386 0.663 0.598 0.667 0.767	16 rs221314 0.075 0.079 0.073 0.158 0.175 0.703 0.99999 0.137	17 rs221307 0.025 0.010 0.018 0.316 0.316 0.462 0.593 0.894 0.452	18 rs6129459 0.060 0.042 0.053 0.311 0.452 0.591 0.889 0.440	19 rs6072018 0.031 0.015 0.028 0.290 0.377 0.579 0.885 0.376	20 rs2425266 0.109 0.095 0.102 0.259 0.376 0.592 0.892 0.376	
(B) No. 1 2 3 4 5 6 7 8 9	Pairwis SNP ID SNP2076 SNP1139 SNP1140 SNP1143 SNP1144 SNP1145 SNP1146	e LD of 2 dbSNP ID rs2425229 rs2425236 rs2425242 rs6124857 rs1275396 rs1275398 rs1275398 rs220079 rs220076	Genes DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 MYL9	Location Intron Intron Intron Intron Intron Intron S-UTR Intron	Pesition 34,440,834 34,445,973 34,452,333 34,484,621 34,501,432 34,501,432 34,503,033 34,540,383 34,540,383 34,556 34,659,971	? locus	1 re2425229 0.986 0.983 0.067 0.009 0.00002 0.033 0.007 0.006	2 rs2425236 1 0.997 0.068 0.010 0.0001 0.033 0.008 0.007	3 rs2425242 0.997 1 0.069 0.010 0.0001 0.033 0.009 0.007	4 rs6124857 0.298 0.298 0.301 0.216 0.128 0.025 0.153 0.173	5 rs1275396 0.096 0.104 0.103 0.524 0.535 0.100 0.580 0.541	6 rs1275404 0.006 0.013 0.013 0.541 0.985 0.183 0.295 0.268	7 rs1275398 0.541 0.556 0.555 0.975 0.982 0.032 0.032	8 ns220079 0.100 0.109 0.110 0.404 0.889 0.852 0.653	9 ns220076 0.092 0.101 0.103 0.436 0.868 0.823 0.619 0.978	10 rs694379 0.086 0.098 0.097 0.407 0.881 0.882 0.653 0.973 0.965	11 rs6071089 0.051 0.069 0.066 0.427 0.946 0.924 1 0.978 0.978	12 rs85440 0.092 0.103 0.100 0.345 0.640 0.838 0.635 0.892 0.890	13 rs555394 0.115 0.113 0.118 0.596 0.850 0.840 0.725 0.955 0.954	14 rs693361 0.134 0.139 0.145 0.589 0.821 0.822 0.712 0.926 0.925	15 rs221311 0.034 0.041 0.042 0.386 0.663 0.598 0.667 0.767 0.751	16 rs221314 0.075 0.079 0.073 0.158 0.175 0.703 0.99999 0.137 0.163	17 rs221307 0.025 0.010 0.018 0.316 0.462 0.593 0.894 0.452 0.452 0.438	18 rs6129459 0.060 0.042 0.053 0.311 0.452 0.591 0.889 0.440 0.421	19 rs6072018 0.031 0.015 0.028 0.290 0.377 0.579 0.885 0.376 0.349	20 rs2425266 0.109 0.095 0.102 0.259 0.376 0.592 0.376 0.892 0.376 0.349	-
No. No. 1 2 3 4 5 6 7 8 9 10	Pairwis SNP ID SNP2076 SNP1139 SNP1140 SNP1143 SNP1144 SNP1145 SNP1145	dbSNP ID rs2425229 rs2425229 rs2425236 rs2425242 rs6124857 rs1275396 rs1275398 rs220079 rs220076 rs29076 rs694379	Genes DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 -	Location Intron Intron Intron Intron Intron Intron S-UTR Intron	ndmark SNF Position 34,440,834 34,445,973 34,462,333 34,462,333 34,462,132 34,501,432 34,503,633 34,540,885 34,659,556 34,659,571 34,622,10	2 locus	1 rs2425229 0.986 0.983 0.067 0.009 0.00002 0.033 0.007 0.006 0.006 0.005	2 rs2425236 1 0.997 0.068 0.010 0.0001 0.033 0.008 0.007 0.007	3 rs2425242 0.997 1 0.069 0.010 0.0001 0.033 0.009 0.007 0.007	4 rs6124857 0.298 0.298 0.301 0.216 0.128 0.025 0.153 0.173 0.155	5 rs1275396 0.096 0.104 0.103 0.524 0.535 0.100 0.580 0.541 0.572	6 rs1275404 0.006 0.013 0.013 0.541 0.985 0.183 0.295 0.268 0.281	7 rs1275398 0.541 0.554 0.555 0.975 0.982 0.032 0.032 0.032	8 rs220079 0.100 0.109 0.404 0.889 0.852 0.653 0.933 0.943	9 rs220076 0.092 0.101 0.103 0.436 0.868 0.823 0.619 0.978 0.919	10 rs694379 0.086 0.097 0.407 0.881 0.882 0.653 0.973 0.969	11 rs6071089 0.051 0.069 0.066 0.427 0.946 0.924 1 0.978 0.946 1	12 rs85440 0.092 0.103 0.100 0.345 0.640 0.838 0.635 0.892 0.890 0.920	13 rs555394 0.115 0.113 0.118 0.596 0.850 0.840 0.725 0.955 0.954 0.954	14 re693361 0.134 0.139 0.145 0.589 0.821 0.822 0.712 0.926 0.925 0.953	15 rs221311 0.034 0.041 0.042 0.386 0.663 0.598 0.667 0.767 0.751 0.779	16 rs221314 0.075 0.079 0.073 0.158 0.175 0.703 0.99999 0.137 0.163 0.119	17 rs221307 0.025 0.010 0.018 0.316 0.462 0.593 0.894 0.452 0.438 0.452 0.438	18 rs6129459 0.060 0.042 0.053 0.311 0.452 0.591 0.452 0.591 0.489 0.440 0.421 0.458	19 rs6072018 0.031 0.015 0.028 0.290 0.377 0.579 0.885 0.376 0.349 0.374	20 rs2425266 0.109 0.095 0.102 0.259 0.376 0.592 0.892 0.376 0.349 0.353	-
No. No. 1 2 3 4 5 6 7 8 9 10 11	Pairwis SNP ID SNP2076 SNP1139 SNP1140 SNP1143 SNP1145 SNP1145	dbSNP ID rs2425229 rs2425239 rs2425236 rs2425242 rs6124867 rs1275396 rs1275596 rs220079 rs220079 rs220076 rs220076	Genes DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 MYL9	Intron Intron Intron Intron Intron Intron Intron Intron 3'-UTR Intron	ndmark SNF Position 34,440,834 34,445,973 34,445,233 34,4462,333 34,454,623 34,450,1432 34,500,333 34,500,333 34,500,333 34,550,333 34,510,333 34,510,333 34,510,333 34,510,333 34,510,333 34,510,333 34,510,333 34,510,335 34,510,359359 34,510,359 34,510,359 34,510,359359 34,510,510,510,510,510,510,510,510,510,510	r ²	1 rs2425229 0.986 0.983 0.067 0.009 0.00002 0.033 0.007 0.006 0.005 0.001	2 rs2425236 1 0.068 0.010 0.0001 0.033 0.008 0.007 0.007 0.003	3 rs2425242 0.997 1 0.069 0.010 0.0001 0.033 0.009 0.007 0.007 0.002	4 rs6124857 0.298 0.298 0.301 0.216 0.128 0.025 0.153 0.173 0.155 0.138	5 xs1275396 0.096 0.104 0.103 0.524 0.535 0.100 0.580 0.541 0.572 0.537	6 rs1275404 0.006 0.013 0.013 0.541 0.985 0.183 0.295 0.268 0.281 0.280	7 rs1275398 0.541 0.554 0.555 0.975 0.982 0.032 0.032 0.032 0.032 0.032	8 ns220079 0.100 0.110 0.404 0.889 0.852 0.653 0.933 0.943 0.943	9 0.092 0.101 0.103 0.436 0.868 0.823 0.619 0.978 0.978 0.919 0.919	10 rs694379 0.086 0.097 0.407 0.881 0.832 0.653 0.973 0.969 0.914	11 rs6071089 0.051 0.069 0.066 0.427 0.946 0.924 1 0.978 0.946 1	12 rs85440 0.092 0.103 0.100 0.345 0.640 0.838 0.635 0.892 0.890 0.920 0.920	13 rs555394 0.115 0.113 0.118 0.596 0.850 0.840 0.725 0.955 0.954 0.954 0.981 0.977	14 re693361 0.134 0.139 0.145 0.589 0.821 0.822 0.712 0.925 0.925 0.925 0.953	15 rs221311 0.034 0.041 0.042 0.386 0.663 0.598 0.667 0.767 0.751 0.779 0.778	16 rs221314 0.075 0.079 0.073 0.158 0.175 0.703 0.99999 0.137 0.163 0.119 0.173	17 rs221307 0.025 0.010 0.018 0.316 0.462 0.593 0.894 0.452 0.438 0.474 0.730	18 rs6129459 0.060 0.042 0.053 0.311 0.452 0.591 0.452 0.591 0.452 0.400 0.421 0.458 0.736	19 rs6072018 0.031 0.015 0.028 0.290 0.377 0.579 0.885 0.376 0.349 0.374 0.618	20 rs2425266 0.095 0.095 0.102 0.259 0.376 0.592 0.376 0.349 0.353 0.353 0.593	- D'l
No. No. 1 2 3 4 5 6 7 8 9 10 11 12	Pairwis SNP ID SNP2076 SNP1139 SNP1140 SNP1143 SNP1145 SNP1145 SNP1147	dbSNP ID rs2425229 rs2425236 rs2425236 rs1245237 rs1275396 rs1275404 rs1275398 rs220079 rs220076 rs6071089 rs6071089 rs6071089 rs6071089	Genes DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4	Intron Intron Intron Intron Intron Intron Intron S-UTR Intron	Position 34,440,834 34,445,973 34,445,233 34,482,233 34,621 34,622 34,625 34,625 34,627 34,	r ²	1 rs2425229 0.986 0.983 0.067 0.009 0.0002 0.033 0.007 0.006 0.005 0.001 0.007	2 rs2425236 1 0.997 0.068 0.010 0.0001 0.033 0.008 0.007 0.007 0.007 0.003 0.009	3 1 0.997 1 0.069 0.010 0.0001 0.033 0.009 0.007 0.007 0.002 0.008	4 rs6124857 0.298 0.298 0.301 0.216 0.128 0.025 0.153 0.173 0.155 0.138 0.111	5 x x 1275396 0.096 0.104 0.103 0.524 0.535 0.100 0.540 0.541 0.570 0.541 0.572 0.537 0.344	6 rs1275404 0.006 0.013 0.013 0.541 0.985 0.183 0.295 0.268 0.281 0.280 0.325	7 rs1275398 0.541 0.556 0.555 0.975 0.982 0.032 0.032 0.029 0.032 0.029 0.032	8 rs220079 0.100 0.109 0.110 0.404 0.889 0.852 0.653 0.933 0.943 0.943 0.776 0.696	9 ns220076 0.092 0.101 0.103 0.436 0.868 0.823 0.619 0.978 0.919 0.742 0.673	10 rs694379 0.086 0.098 0.097 0.407 0.881 0.832 0.653 0.973 0.969 0.814 0.814	11 rs6071089 0.051 0.069 0.066 0.427 0.946 0.924 1 0.978 0.946 1 0.9597	12 rs85440 0.092 0.103 0.100 0.345 0.640 0.838 0.635 0.892 0.892 0.890 0.920 0.917	13 rs555294 0.115 0.113 0.118 0.596 0.850 0.840 0.725 0.955 0.955 0.954 0.981 0.981 0.989	14 rs693361 0.134 0.139 0.145 0.899 0.821 0.822 0.712 0.926 0.953 0.953 0.944 0.976	15 rs221311 0.034 0.041 0.042 0.386 0.663 0.598 0.667 0.767 0.751 0.779 0.778 0.778	16 rs221314 0.075 0.079 0.073 0.168 0.175 0.703 0.99999 0.137 0.163 0.119 0.173 0.160	17 ns221307 0.025 0.010 0.018 0.316 0.462 0.593 0.894 0.452 0.452 0.452 0.452 0.452 0.452 0.452 0.452 0.452 0.452 0.452 0.452 0.452 0.452 0.553 0.553 0.553 0.553 0.553 0.555 0.	18 rs6129459 0.060 0.042 0.053 0.311 0.452 0.591 0.489 0.440 0.421 0.425 0.736 0.502	19 rs6072018 0.031 0.015 0.028 0.290 0.377 0.885 0.376 0.349 0.374 0.618 0.437	20 rs2425266 0.109 0.095 0.102 0.259 0.376 0.592 0.376 0.349 0.353 0.593 0.416	- D'l
(B) No. 1 2 3 4 5 6 7 8 9 10 11 12 13 13	Pairwis SNP ID SNP2076 SNP1139 SNP1140 SNP1143 SNP1145 SNP1145 SNP1145 SNP1145 SNP1147 SNP1148	dbSNP ID rs2425229 rs2425229 rs2425236 rs1245236 rs1275396 rs1275396 rs1275398 rs220079 rs220076 rs854379 rs854030 rs8553394	Genes DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 -	Intron Intron Intron Intron Intron Intron Intron Intron Intron Intron Intron	undmark SNF Pesition 34,440,834 34,446,973 34,446,973 34,462,973 34,462,133 34,653,533 34,650,533 34,650,571 34,662,910 34,617,113 34,617,753	r ²	s 1 rs2425229 0.988 0.983 0.007 0.009 0.009 0.0002 0.033 0.007 0.006 0.005 0.007 0.007 0.007	2 rs2425236 1 0.997 0.068 0.010 0.0001 0.033 0.008 0.007 0.007 0.003 0.009 0.009	3 rs2425242 0.997 1 0.069 0.010 0.0001 0.033 0.009 0.007 0.007 0.002 0.008	4 rs6124857 0.298 0.298 0.216 0.128 0.025 0.153 0.173 0.155 0.138 0.111 0.151	5 xs1275396 0.096 0.104 0.103 0.524 0.535 0.100 0.580 0.541 0.580 0.541 0.572 0.572 0.374 0.390	6 rs1275404 0.006 0.013 0.013 0.541 0.985 0.183 0.295 0.268 0.268 0.280 0.325 0.694	7 rs1275398 0.541 0.555 0.975 0.982 0.032 0.032 0.032 0.032 0.032 0.035 0.035 0.035	8 rs220079 0.100 0.109 0.110 0.404 0.889 0.852 0.653 0.933 0.943 0.943 0.944 0.946 0.9	9 ns220076 0.092 0.101 0.103 0.436 0.868 0.823 0.619 0.978 0.919 0.742 0.673 0.354	10 rs694379 0.086 0.098 0.407 0.881 0.832 0.653 0.973 0.969 0.814 0.738 0.382	11 rs6071089 0.051 0.069 0.066 0.427 0.946 0.924 1 0.978 0.946 1 0.597 0.305	12 rs85440 0.092 0.103 0.100 0.345 0.640 0.838 0.635 0.892 0.890 0.920 0.917 0.917	13 rs555294 0.115 0.113 0.118 0.596 0.850 0.840 0.725 0.955 0.955 0.954 0.981 0.977 0.989	14 rs693361 0.134 0.139 0.145 0.829 0.821 0.822 0.712 0.925 0.925 0.925 0.925 0.953 0.954 0.976	15 rs221311 0.034 0.041 0.042 0.386 0.663 0.598 0.667 0.767 0.779 0.778 0.779 0.778 0.789 0.735	16 rs221314 0.075 0.079 0.073 0.168 0.175 0.703 0.99999 0.137 0.163 0.119 0.173 0.180 0.656	17 rs221307 0.025 0.010 0.018 0.316 0.462 0.452 0.452 0.452 0.458 0.452 0.458 0.474 0.730 0.512 0.629	18 rs6129459 0.060 0.042 0.053 0.311 0.452 0.591 0.489 0.440 0.421 0.421 0.458 0.736 0.502 0.621	19 rs6072018 0.031 0.015 0.028 0.290 0.377 0.579 0.885 0.376 0.349 0.374 0.618 0.437 0.608	20 rs2425266 0.109 0.095 0.259 0.376 0.592 0.376 0.389 0.376 0.349 0.353 0.593 0.416 0.620	- D'l
No. No. No. No. No. No. No. No.	Pairwis SNP ID SNP2076 SNP1139 SNP1140 SNP1143 SNP1145 SNP1145 SNP1145 SNP1145 SNP1145 SNP1145 SNP1145	e LD of 2 dbSNP ID rs2425229 rs2425239 rs4242036 rs1275396 rs1275404 rs1275396 rs1275404 rs1275509 rs220079 rs220079 rs220079 rs82340 rs555394	Genes DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 TGIF2 C200rf24 SLA2	Iround a la Location Intron Intron Intron Intron Intron Intron Intron Intron Intron Intron	ndmark SNF Position 34,440,834 34,440,834 34,450,973 34,452,333 34,452,333 34,452,333 34,450,432 34,450,432 34,450,835 34,589,556 34,650,971 34,645,765 34,657,739 34,687,759 34,587,759 34,587,7	r ²	3 1 rs2425229 0.988 0.983 0.067 0.0002 0.033 0.007 0.006 0.001 0.007 0.007 0.007 0.007 0.007	2 rs2425236 0.997 0.068 0.010 0.000 0.000 0.007 0.003 0.007 0.003 0.007 0.003	3 rs2425242 0.997 1 0.069 0.010 0.0001 0.033 0.009 0.007 0.007 0.002 0.008 0.011	4 rs6124857 0.298 0.298 0.301 0.216 0.128 0.025 0.153 0.173 0.155 0.138 0.1131 0.151 0.144	$\frac{5}{181275396}$ 0.096 0.104 0.103 0.524 0.535 0.100 0.580 0.541 0.572 0.537 0.344 0.3390 0.382	6 vs1275404 0.006 0.013 0.013 0.385 0.385 0.285 0.286 0.281 0.280 0.325 0.428 0.325 0.4284 0.448	7 rs1275398 0.541 0.554 0.555 0.975 0.982 0.032 0.032 0.032 0.032 0.032 0.032 0.061 0.035 0.102	8 rs220079 0.100 0.109 0.110 0.408 0.852 0.653 0.933 0.943 0.776 0.693 0.352	9 rst220076 0.092 0.101 0.103 0.436 0.823 0.619 0.919 0.919 0.742 0.619 0.913 0.354 0.324	10 rs694379 0.096 0.098 0.097 0.407 0.881 0.832 0.653 0.973 0.969 0.814 0.384 0.382 0.351	11 rs6071089 0.051 0.069 0.066 0.427 0.946 0.924 1 0.978 0.946 1 0.597 0.305 0.277	12 rs85440 0.092 0.103 0.100 0.345 0.640 0.838 0.635 0.892 0.890 0.920 0.917 0.917	13 7s555394 0.115 0.113 0.113 0.113 0.113 0.596 0.850 0.840 0.725 0.955 0.955 0.954 0.981 0.977 0.989	14 vre693361 0.134 0.139 0.145 0.821 0.822 0.712 0.926 0.925 0.953 0.944 0.976 0.970	15 rs221311 0.034 0.041 0.042 0.366 0.663 0.598 0.667 0.767 0.761 0.778 0.778 0.788 0.785 0.735	16 rs221314 0.075 0.079 0.073 0.168 0.175 0.103 0.183 0.119 0.173 0.163 0.199 0.173 0.666 0.675	17 rs221307 0.025 0.010 0.018 0.316 0.462 0.593 0.452 0.458 0.452 0.458 0.474 0.730 0.512 0.629 0.657	18 rs6129459 0.060 0.042 0.053 0.314 0.591 0.452 0.591 0.452 0.421 0.458 0.736 0.536 0.536 0.536 0.531 0.649	19 rx6072018 0.031 0.015 0.028 0.290 0.377 0.879 0.377 0.349 0.374 0.349 0.374 0.374 0.437 0.618 0.437 0.608	20 rs2425266 0.109 0.095 0.259 0.376 0.592 0.376 0.389 0.376 0.349 0.353 0.593 0.416 0.620 0.671	- D'l
(B) No. 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 14	Pairwis SNP ID SNP2076 SNP1139 SNP1140 SNP1143 SNP1144 SNP1145 SNP1145 SNP1145 SNP1147 SNP1147 SNP1148 SNP2081 SNP2081	e LD of 2 dbSNP ID rs2425229 rs2425236 rs2425242 rs6124657 rs1275308 rs220076 rs520076 rs520076 rs5694379 rs60371089 rs653394 rs653394 rs653394	Genes DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 TGIP2 C200rf24 SLA2 NDRG3	Iround a la Location Intron Intron Intron Intron Intron Intron Intron Intron Intron Intron Intron Intron	Position 34,440,834 34,446,933 34,440,933 34,454,933 34,454,933 34,454,933 34,454,933 34,540,933 34,650,971 34,612,910 34,617,113 34,645,765 34,670,739 34,645,087 34,670,739 34,670,740 34,710,770 35	r ²	3 1 rs2425229 0.986 0.983 0.067 0.009 0.0002 0.033 0.007 0.006 0.001 0.007 0.007 0.007 0.007	2 rs2425236 1 0.997 0.068 0.010 0.033 0.008 0.007 0.003 0.007 0.007 0.003 0.009 0.007 0.010	3 rs2425242 0.997 1 0.069 0.010 0.0001 0.003 0.009 0.007 0.007 0.007 0.007 0.002 0.008 0.011 0.001	4 rs6124857 0.298 0.298 0.301 0.216 0.128 0.025 0.153 0.173 0.155 0.138 0.1111 0.151 0.144 0.066	5 rs1275396 0.096 0.104 0.103 0.524 0.535 0.100 0.580 0.541 0.572 0.537 0.344 0.342 0.342 0.342	6 rs1275404 0.006 0.013 0.013 0.541 0.985 0.285 0.288 0.281 0.280 0.325 0.648 0.349	7 rs1275398 0.541 0.554 0.555 0.975 0.982 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.032	8 rs220079 0.100 0.109 0.110 0.404 0.859 0.653 0.933 0.943 0.776 0.696 0.332 0.332 0.245	9 rs220076 0.092 0.101 0.103 0.436 0.882 0.882 0.883 0.619 0.978 0.978 0.919 0.742 0.673 0.324 0.324	10 rs694379 0.086 0.097 0.407 0.832 0.653 0.973 0.969 0.969 0.914 0.738 0.814 0.738 0.82 0.351	11 rs6071089 0.051 0.069 0.066 0.427 0.944 1 0.978 0.946 1 0.597 0.305 0.277 0.203	12 rs85440 0.092 0.103 0.100 0.345 0.640 0.838 0.838 0.835 0.892 0.890 0.920 0.917 0.442 0.419 0.296	13 re555394 0.115 0.113 0.118 0.596 0.850 0.840 0.725 0.955 0.954 0.981 0.977 0.989 0.914	14 re693361 0.134 0.139 0.145 0.589 0.822 0.712 0.925 0.925 0.953 0.944 0.976 0.970	15 rs221311 0.034 0.041 0.042 0.386 0.663 0.588 0.667 0.767 0.751 0.779 0.778 0.778 0.778 0.778 0.735 0.770	16 rs221314 0.075 0.073 0.158 0.175 0.703 0.188 0.175 0.703 0.199999 0.137 0.183 0.119 0.173 0.180 0.156 0.656 0.6575 0.604	17 18221307 0.025 0.010 0.018 0.316 0.462 0.583 0.894 0.452 0.452 0.452 0.438 0.474 0.730 0.512 0.657 0.810	18 rs6129459 0.060 0.042 0.053 0.311 0.452 0.591 0.452 0.591 0.452 0.591 0.452 0.591 0.452 0.591 0.452 0.592 0.621 0.626	19 rs6072018 0.031 0.028 0.290 0.377 0.879 0.886 0.376 0.374 0.618 0.349 0.374 0.618 0.437 0.608 0.437	20 rs2425266 0.109 0.955 0.102 0.259 0.356 0.369 0.376 0.349 0.353 0.593 0.416 0.621 0.601 0.603	- D'l
(B) No. 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16	Pairwis SNP ID SNP2076 SNP1139 SNP1140 SNP1143 SNP1145 SNP1145 SNP1145 SNP1145 SNP1145 SNP1145 SNP1147 SNP1148 SNP2081 SNP2082 SNP2082	e LD of 2 dbSNP ID rs2425229 rs2425236 rs2425242 rs6124857 rs1275396 rs1275496 rs220079 rs8220079 rs8270079 rs85440 rs65071099 rs85440 rs655394 rs8543031 rs823131	USNPs a Genes DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 TGIP2 C20orf24 SLA2 NDRG3	Iround a la Location Intron Intron Intron Intron Intron S-UTR Intron Intron Intron Intron Intron	redition	r ²	3 1 rs2425229 0.986 0.986 0.988 0.067 0.009 0.0002 0.033 0.007 0.005 0.001 0.007 0.007 0.007 0.007 0.007 0.000 0.007	2 rs2425236 1 0.997 0.068 0.010 0.008 0.007 0.008 0.007 0.003 0.009 0.007 0.001 0.001	3 rs2425242 0.997 1 0.069 0.010 0.0001 0.003 0.007 0.007 0.002 0.008 0.008 0.008 0.008 0.011 0.003	4 rs6124857 0.298 0.298 0.298 0.298 0.216 0.128 0.125 0.153 0.155 0.155 0.155 0.138 0.111 0.151 0.151 0.144 0.006	5 xs1275396 0.096 0.104 0.103 0.524 0.535 0.100 0.580 0.580 0.580 0.580 0.580 0.572 0.537 0.344 0.390 0.352 0.342 0.342 0.342 0.344 0.390 0.352 0.344 0.390 0.352 0.344 0.390 0.352 0.344 0.390 0.352 0.344 0.352 0.352 0.357 0.354 0.537 0.344 0.352 0.537 0.344 0.352 0.352 0.537 0.354 0.537 0.537 0.354 0.537 0.537 0.354 0.537 0.537 0.354 0.537 0.354 0.537 0.354 0.537 0.354 0.537 0.354 0.537 0.354 0.537 0.354 0.355 0.537 0.344 0.3552 0.354 0.3552 0.355 0.357 0.354 0.3552 0.355 0.355 0.357 0.3552 0.3552 0.3552 0.3557 0.3552 0.35552 0.35552 0.3555 0.3555 0.3555 0.35555 0.35555	6 rs1275404 0.006 0.013 0.013 0.411 0.985 0.285 0.295 0.281 0.280 0.325 0.694 0.648 0.345	7 rs1275398 0.541 0.555 0.975 0.982 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.041	8 ns220079 0.100 0.100 0.404 0.889 0.852 0.653 0.933 0.943 0.943 0.943 0.943 0.943 0.943 0.943 0.943 0.943 0.943 0.944 0.945 0.55	9 0.092 0.101 0.436 0.823 0.823 0.819 0.978 0.919 0.978 0.919 0.742 0.673 0.354 0.324 0.321 0.007	10 rs694379 0.086 0.097 0.407 0.832 0.653 0.973 0.969 0.814 0.738 0.382 0.382 0.382 0.382 0.382 0.381 0.382	11 rs6071089 0.051 0.069 0.066 0.427 0.946 0.924 1 0.978 0.978 0.9597 0.305 0.277 0.205 0.277 0.205	12 rs85440 0.092 0.103 0.100 0.345 0.640 0.640 0.838 0.635 0.892 0.890 0.920 0.917 0.442 0.419 0.296 0.006	13 re555394 0.115 0.113 0.596 0.850 0.840 0.725 0.955 0.955 0.955 0.981 0.977 0.989 0.914 0.514 0.5914	14 re693361 0.134 0.139 0.145 0.889 0.821 0.822 0.712 0.926 0.925 0.953 0.944 0.976 0.970 0.542 0.047	15 rs221311 0.034 0.041 0.042 0.386 0.663 0.598 0.667 0.767 0.767 0.779 0.778 0.779 0.778 0.789 0.735 0.770	16 rs221314 0.075 0.079 0.035 0.135 0.703 0.137 0.133 0.19 0.173 0.160 0.656 0.675 0.604	17 rs221307 0.025 0.010 0.018 0.316 0.452 0.533 0.452 0.452 0.438 0.474 0.730 0.512 0.629 0.659 0.639	18 rs6129459 0.060 0.042 0.053 0.311 0.452 0.591 0.440 0.421 0.448 0.736 0.502 0.621 0.649 0.869 0.869 0.661	19 rs6072018 0.031 0.015 0.290 0.377 0.579 0.885 0.376 0.376 0.374 0.618 0.437 0.608 0.437 0.608 0.656 0.760	20 rs2425266 0.109 0.955 0.102 0.259 0.376 0.376 0.349 0.353 0.593 0.416 0.620 0.671 0.808 0.808	- D'l
No. No. No. No. No. No. No. No.	Pairwis SNP ID SNP2076 SNP1139 SNP1140 SNP1143 SNP1144 SNP1145 SNP145 SNP1	se LD of 2 dbSNP ID rs2425229 rs2425236 rs2425242 rs6124536 rs1275404 rs1275404 rs1275404 rs1275404 rs1275404 rs220076 rs20076 rs20071089 rs55334 rs55334 rs25334 rs221311 rs221311	USNPs a Genes DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 DLGAP4 TGIF2 C20orf24 SLA2 NDRG3 NDRG3	Iround a la Location Intron Intron Intron Intron Intron JINTON JINTON Intron Intron Intron Intron Intron Intron Intron	undmark SNF Pusition 04.440,334 04.440,333 04.440,233 04.440,233 04.440,233 04.440,233 04.440,233 04.450,233 04.503,033 04.503,033 04.503,033 04.503,705 04.617,113 04.617,715 04.617,715 04.617,715 04.617,715 04.617,715 04.617,715 04.617,715 04.617,715 04.719,874 04.719,874 04.719,874 04.719,874 04.729,875 04.719,874 04.729,875 04.719,874 04.729,875 04.719,874 04.729,875 04.719,874 04.729,875 04.719,874 04.729,875 04.719,874 04.729,875 04.719,874 04.729,875 04.719,874 04.729,875 04.719,874 04.729,875 04.719,874 04.729,875 04.719,874 04.729,875 04.719,874 04.729,875 04.729,8	r ²	1 12425229 0.986 0.983 0.067 0.009 0.0003 0.003 0.005 0.005 0.005 0.007 0.006 0.007 0.007 0.007 0.007 0.007 0.007 0.007	2 rs2425236 1 0.997 0.068 0.010 0.003 0.007 0.007 0.007 0.009 0.007 0.010 0.001 0.001 0.001 0.0001	3 rs2425242 0.997 1 0.069 0.010 0.0001 0.033 0.009 0.007 0.007 0.007 0.007 0.007 0.002 0.008 0.010 0.008 0.011 0.008 0.011 0.008 0.010 0.000 0.010 0.000 0.000 0.010 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0.0000 0	4 rs6124857 0.298 0.298 0.298 0.298 0.298 0.216 0.128 0.025 0.153 0.153 0.153 0.153 0.173 0.155 0.138 0.111 0.151 0.144 0.066 0.0058	5 rs1275396 0.096 0.104 0.103 0.524 0.535 0.100 0.580 0.541 0.572 0.541 0.572 0.344 0.390 0.352 0.248 0.028	6 rs1275404 0.006 0.013 0.013 0.541 0.985 0.295 0.268 0.280 0.268 0.280 0.325 0.694 0.448 0.349 0.054	7 rs1275398 0.541 0.556 0.555 0.975 0.982 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.032 0.035 0.035 0.102 0.102 0.083 0.083 0.012	8 0.100 0.101 0.102 0.110 0.404 0.889 0.853 0.653 0.933 0.943 0.764 0.933 0.943 0.764 0.933 0.943 0.764 0.933 0.943 0.764 0.933 0.943 0.764 0.943 0.764 0.943 0.764 0.363 0.364 0.363 0.364 0.365 0.365 0.365 0.365 0.935	9 	10 19694379 0.086 0.097 0.407 0.881 0.653 0.653 0.973 0.973 0.969 0.514 0.351 0.382 0.381 0.382 0.351 0.252 0.040	11 rs6071089 0.051 0.069 0.066 0.427 0.946 1 0.978 0.978 1 0.597 0.305 0.277 0.305 0.277 0.203 0.010	12 rs85440 0.992 0.103 0.103 0.345 0.640 0.838 0.635 0.892 0.890 0.920 0.920 0.917 0.442 0.419 0.296 0.0183	13 rs555394 0.113 0.113 0.13 0.596 0.800 0.725 0.954 0.954 0.954 0.981 0.977 0.989 0.914 0.512 0.051	14 re693361 0.134 0.139 0.145 0.829 0.821 0.822 0.712 0.925 0.925 0.944 0.976 0.970 0.542 0.0542 0.033	15 rs221311 0.034 0.041 0.041 0.386 0.663 0.598 0.667 0.767 0.767 0.779 0.779 0.779 0.778 0.789 0.735 0.770	16 rs221314 0.075 0.073 0.168 0.175 0.703 0.168 0.175 0.137 0.163 0.119 0.156 0.656 0.656 0.656 0.654 0.061	17 rs221307 0.025 0.010 0.018 0.316 0.462 0.593 0.452 0.552 0.	18 rs6129459 0.060 0.042 0.053 0.311 0.452 0.452 0.452 0.454 0.464 0.458 0.766 0.502 0.621 0.649 0.506 0.611 0.997	19 rs6072018 0.015 0.028 0.290 0.377 0.885 0.376 0.349 0.374 0.374 0.374 0.374 0.437 0.608 0.437 0.656 0.796 0.987	20 rs2425266 0.109 0.095 0.229 0.376 0.592 0.892 0.376 0.593 0.416 0.620 0.671 0.803 0.906	- D'l
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Table 5 Pairwise LD values between SNPs, as defined by |D'| and r^2 around the HNF4 α locus (A) and a landmark SNP locus (B) (A) Pairwise LD of 17 SNPs around the HNF4 α locus

Haplotype blocks I Haplotype block r²

Results of genotyping data using 725 Japanese samples (367 cases and 358 controls). Each column shows the values of linkage disequilibrium (LD) between pairwise SNPs: |D'| (upper right triangle) and r (lower left triangle). Blue coloring indicates values over 0.95, light blue indicates values ranging from 0.95 and 0.90, and light green indicates values ranging from 0.90 to 0.85. Based on the values of |D'| and r^2 , inferred haplotype block(s) are shown as a gray scale bar at the bottom of each table. SNPs with the nominal P < 0.05 by a single-marker association test are in boldface

Selection of a landmark SNP with an extensive association study

In the first association test, we used a total of 1,044 SNPs markers, consisting of 581 TaqMan assays and 463 custom-designed SNPs assays for analysis (Supplementary Table 4). After the first association test, we found a significant association (P < 0.05) with 142 SNPs markers (13.6%) in at least one of the four chi-square tests (Table 3).

Subsequently, the initial 142 candidate SNPs were genotyped in the second test samples (independent samples) with the number larger than the first test samples. The genotyping data from each association test were combined and calculated. Of these 142 SNPs, SNP1146 (rs220076) was mostly associated with type 2 diabetes in four chi-square tests (P = 0.00231, 0.01010, 0.01157, and 0.01507; allele model) (Table 4B). The unadjusted odds ratio for SNP1146 (rs220076) was 1.23 (95% confidence interval 1.077–1.399). Logistic

regression analysis indicated that the association with SNP1146 (rs220076) remained statistically significant after adjusting for age, sex, and BMI (P = 0.002). The same result was obtained when statistical significance was assessed with two independent samples, indicating that SNP1146 (rs220076) was a putative susceptibility variant.

With the large number of SNPs examined, it was necessary to exclude false-positive results by multiple testing. We applied standard Bonferroni's correction for multiple testing (corrected for 1,044 tests), but the association with SNP1146 (rs220076) was no longer significant. In addition, we calculated the FDR with 142 SNPs in the second association test as a threshold value of under 0.1 (Shiffman et al. 2005). Unfortunately, among 142 SNPs, there was no SNP under this threshold (Supplementary Fig. 2. and Supplementary Table 5). Taken together, the magnitude of the nominal P value was statistically rather weak after the evaluation of multiple testing, in

Haplotype rs2273 C/T										
C/T	i18 rs6073435	rs6031601	Frequency (1	Bayesian methe	(pc	Frequency (EM algorithm)		P value	Permutation
	A/T	C/A	Overall $(n = 1,818)$	$\begin{array}{l} \text{Control} \\ (n = 893) \end{array}$	Case $(n = 925)$	Overall $(n = 1,818)$	Control $(n = 893)$	Case $(n = 925)$		P value
#1 T	A	A	0.602	0.618	0.586	0.602	0.618	0.586	0.0403	0.0438
#2 C	Т	C	0.328	0.312	0.343	0.328	0.311	0.343	0.0403	0.0436
#3 C	A	C	0.033	0.034	0.033	0.033	0.034	0.033	0.92	0.93
#4 C	T	A	0.032	0.032	0.033	0.033	0.032	0.033	0.86	0.93
#5 T	А	C	0.005	0.005	0.005	0.005	0.005	0.005	0.69	0.82
#6 C	А	A	< 0.001	< 0.001	< 0.001					
#7 T	Τ	C								
#8 T	Т	A								
(B) Landmark SNP lo	suc									
Haplotype rs2200	79 rs220076	rs694379	Frequency (Ba	yesiian methoc	1)	Frequency (E	M algorithm)		P value	Permutation
G/A	C/A	C/T	Overall	Control	Case	Overall	Control	Case		P value
1			(n = 1,818)	(n = 893)	(n = 925)	(n = 1, 818)	(n = 893)	(n = 925)		
#1 G	C	С	0.531	0.556	0.507	0.531	0.555	0.508	0.0044	0.0047
#2 A	A	Т	0.443	0.418	0.467	0.444	0.418	0.469	0.0023	0.0028
#3 G	А	C	0.012	0.010	0.013	0.011	0.010	0.012	0.59	0.58
#4 G	С	Τ	0.006	0.006	0.005	0.005	0.006	0.005	0.76	0.72
#5 A	С	C	0.004	0.004	0.004	0.004	0.004	0.004	0.95	0.87
#6 G	А	Τ	0.002	0.003	0.001	0.002	0.003	0.001	0.14	0.12
#7 A	A	C	0.002	0.002	0.001	0.002	0.002	0.001	0.37	0.29
#8 A	С	Т	0.001	0.001	0.001	0.001	0.001	0.001	0.55	0.60

Fig. 2 a Map of chromosome 20, STS and 1,044 SNPs markers (after quality control). Blue bars indicate 581 SNPs marker (TaqMan SNP Genotyping Assays) positions. Red bars indicate additional custom 463 SNPs marker positions, especially designed for this study. Black bars show two STS marker positions. **b** Results of *P* value plots by allele frequency chisquare test with 142 SNPs using 925 cases and 893 controls. Asterisk indicates SNP1146 (rs220076) with the most significant P value (P = 0.0023) as a landmark SNP. Red lines indicate reference P values (P = 0.1and 0.01, respectively). a and **b** are illustrated to the same physical scale. The horizontal axis shows the physical position on chromosome 20q based on NCBI Build 35 human genome



which the selected variant could not reach the statistical confidence level.

Searching around a landmark SNP

As a landmark, SNP1146 (rs220076) was mapped to the first intron of MYL9 (myosin light polypeptide 9, regulatory: NCBI Gene ID; 10398). Four additional SNPs markers (rs1275404, rs1275398, rs694379, and rs6071089) were selected from the public database and placed around SNP 1146 (rs220076) for fine analysis (Table 4B). SNP1145 (rs220079) and rs694379 adjacent to the landmark SNP also revealed association. SNP1145 (rs220079) was mapped to the 3'-UTR (untranslated region) of DLGAP4 (discs, large [Drosophila] homolog-associated protein 4: Gene ID; 22839).

With the re-sequencing method, we potentially searched for the additional variants in two genes, MYL9 (full length, 10 kb) and DLGAP4 (exons 10, 11, 12, and 3'-UTR, 8 kb). We discovered 13 novel SNPs that were not registered in the public database, but there were no common SNPs (MAF > 0.15) or functional variants (data not shown). Consequently, all novel SNPs were considered to be rare and synonymous variants, and were excluded from further analysis.

By two pairwise measurements of LD (|D'| and r^2), the property of LD was examined around the landmark

SNP (Table 5B). In the interpretation of r^2 , there was one LD block with extremely high correlation (range from 0.919 to 0.943) among three common SNPs including a landmark SNP. Because LD did not extend outside the region by the interpretation of r^2 , one haplotype block was predicted to consist of three common SNPs (rs220079, rs220076, and rs694379).

Haplotype-based association test including landmark SNP

We examined the haplotype-based test by a multi-locus approach, which was a more sensitive method for detecting associations than the assessment of individual SNPs. In the interpretation of r^2 , one haplotype block was recognized to show a clear transition zone, which was 23 kb in full length including the landmark SNP (Table 6B). When haplotype frequencies were estimated with both Bayesian method and EM algorithm, two common haplotypes were observed to cover more than 95% data. Because there was no major difference in the results with two methods, the haplotype phase was apparent.

In each haplotype analysis, #1 haplotype was inferred as a protective haplotype, and #2 haplotype was inferred as a risk haplotype. However, the magnitudes of the nominal P values (0.0044 and 0.0023) were weak after Bonferroni's correction (corrected for 1,044 plus eight tests), suggesting that there was no significant difference in each haplotype.

Discussion

Although a large quantity of information about the human genome is now available in genetic studies, tremendous efforts have been made to detect genetic polymorphisms contributing to the susceptibility to common complex human diseases. Because complex diseases are caused by several interacting genetic determinants, most of which have small effects, it is difficult to clearly identify the susceptibility locus (Wang et al. 2005; Hirschhorn and Daly 2005; Togawa et al. 2006). However, an association study using common variants has been proposed as a powerful strategy to search for genuine variants that underlie complex traits. With intensive association studies, common variants have important roles in common complex diseases, such as rheumatoid arthritis (Suzuki et al. 2003), ischemic heart disease (Ozaki et al. 2002), inflammatory bowel disease (Hugot et al. 2001), and diabetes mellitus (Grant et al. 2006). Under the current tendency, we designed an extensive association study and succeeded in identifying the susceptibility gene(s) and variant(s) related to common complex diseases as previously reported (Hamada et al. 2005; Kato et al. 2006).

Type 2 diabetes is a complex disease influenced by multiple environmental and genetic factors, and a number of potential loci have been proposed by wholegenome linkage analysis. In particular, several groups have focused on chromosome 20q, in which evidence of linkage was shown in the same region in different populations (Ghosh et al. 1999, 2000; Vionnet et al. 2000; Permutt et al. 2001; Duggirala et al. 2001; Luo et al. 2001; Mori et al. 2002; Iwasaki et al. 2003). It was suggested that chromosome 20q was a promising locus including certain susceptibility variant(s) or gene(s) with type 2 diabetes. In this study, we regarded HNF4 α as a candidate gene with type 2 diabetes to undertake a detailed association study. Simultaneously, to reveal whether other variant(s) were associated with disease on chromosome 20q11.21-13.13, a total of 1,044 SNPs were extensively examined in 1,818 Japanese samples. In part of all the tests, the nominal P values were weakly associated with disease. Although we could not completely rule out the possibility of multiple and minor genetic contributions on this locus, we propose that these results are statistically non-significant findings after adjusting strictly for multiple testing.

There are several explanations for our inability to detect the disease-susceptibility variant with a statistical confidence level. First, it is possible that the initial linkage studies might overestimate chromosome 20q as a candidate locus. This hypothesis seems most likely since a recent Japanese study failed to confirm the previous linkage evidence on chromosome 20q. Similarly, in Chinese, two recent reports indicated a lack of evidence for previous linkage on chromosome 20q (Xiang et al. 2004; Ng et al. 2004). In addition, several groups have regarded HNF4 α as a disease susceptibility gene due to its chromosomal location under a linkage peak and extensively searched for susceptibility variants (Winckler et al. 2005; Bagwell et al. 2005; Silander et al. 2004; Love Gregory et al. 2004; Vaxillaire et al. 2005). However, in an association study involving more than 7,000 subjects, HNF4 α variants showed a complete lack of confirmation of disease susceptibility (Winckler et al. 2005). We also propose that variants in the HNF4 α gene have no major effect on the risk of disease in Japanese. Despite successful cases (Grant et al. 2006), it will be difficult to narrow down the regions of linkage evidence for type 2 diabetes (Permutt et al. 2002, 2005).

A second possibility is that our results are false negative (type 2 error). In the setting of an association study, several factors could cause us to miss the true variant. It is likely that we lacked the power to detect a subtle association due to relatively small sample numbers in the first association test (380 vs.380 samples). With prior simulation, our samples (1,818 samples) were sufficient to detect the susceptibility locus with a power less than 80% as the maximum. Nevertheless, a more powered study involving thousands of subjects may uncover the genuine susceptibility variant(s), because it is predicted that true variant(s) have only a modest effect on type 2 diabetes. However, it will be a laborious procedure to collect enough late onset type 2 diabetes samples, making it difficult to replicate the findings.

As the major weak point, controls were unmatched with cases for age. We suppose the interpretation to be reliable since logistic regression analysis showed a significant association after adjusting for age. However, this analysis could not be entirely correct because of the sampling bias. If the power is affected by bias and is less than the value with prior simulation, it is possible to overlook the genuine association on the 20q locus. Within the framework of this study, it was difficult to evaluate the actual effect of sampling bias, even if it was present. Thus, as one favored approach, it is proposed to confirm the results with other independent populations, such as controls in a large cohort study (Hattersley and McCarthy 2005).

We supposed that marker density must be relatively dense to completely avoid false negatives. It was not possible to place SNPs markers at less than 10-kb intervals due to the experimental cost and time, but our approach is expected to ensure a practical solution (Hamada et al. 2005; Kato et al. 2006). Additionally, an unknown causal variant was clearly detected with this approach using evenly spaced SNPs markers at 8–5 kb density (Edwards et al. 2005).

It is also proposed that an evenly spaced marker might not be a good guide for the design of an association study, because there is massive variability in the extent of LD that overstates or understates the useful range (Reich et al. 2002). To elucidate this question, the HapMap project established the generality of human haplotypes in multiple populations. Unfortunately, the HapMap resource was not completely available at the onset of this study, and it was necessary to design a different strategy, as presented here.

Furthermore, it was supposed that Bonferroni's correction would be too conservative and would likely to lead to a false negative result, because SNPs were not independent due to strong LD. To examine this point, the number of LD blocks was calculated by the definition of |D'|, which indicated that the target region contained 207 LD blocks (unpublished data). However, to evaluate Bonferroni's correction with the number of LD blocks (i.e., 0.05 divided by the number of LD blocks), the nominal P value with a landmark SNP did not reach the statistical confidence level.

The other possibility is that there is heterogeneity among different populations. For instance, the allele frequencies of some variants in HNF4 α are quite different between Caucasians and Asians. The G allele for rs1884613 had a frequency of 0.19 in HapMap CEU, which showed a causative variant in the initial two reports (Love Gregory et al. 2004; Silander et al. 2004). In contrast, the same allele had a different frequency of 0.47, indicating a lack of association in our study. A difference in allele frequencies between populations may suggest specific population-attributed risks of type 2 diabetes. Hence, our observations could not completely rule out evidence of association on chromosome 20q in other ethnic groups. To explain this hypothesis, an additional proof of heterogeneity should be documented.

In this study, we presented a systematic gene-based and two-stage association study for the assessment of multiple markers across a relatively long range on chromosome 20q, and showed novel data within the framework. In spite of the initial notable prediction with linkage evidence, our results are lacking in evidence that certain SNPs had a strong effect on the risk of type 2 diabetes within the target region. Under these association study conditions, it is likely that many reasonably powered studies would produce negative results, which do not attract attention due to publication bias (Blomqvist et al. 2006; Freimer and Sabatti 2005). However, we are convinced that these data could help to resolve the important role of variant(s) on human chromosome 20q.

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