Genome-Wide Search for Type 2 Diabetes in Japanese Affected Sib-Pairs Confirms Susceptibility Genes on 3q, 15q, and 20q and Identifies Two New Candidate Loci on 7p and 11p

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The genetic background that predisposes the Japanese population to type 2 diabetes is largely unknown. Therefore, we conducted a 10-cM genome-wide scan for type 2 diabetes traits in the 359 affected individuals from 159 families, yielding 224 affected sib-pairs of Japanese origin. Nonparametric multipoint linkage analyses performed in the whole population showed one suggestive linked region on 11p13-p12 (maximum logarithm of odds score [MLS] 3.08, near Pax6) and seven potentially linked regions (MLS >1.17) at 1p36-p32, 2q34, 3q26-q28, 6p23, 7p22-p21, 15q13-q21, and 20q12-q13 (near the gene for hepatocyte nuclear factor-4α [HNF-4α]). Subset analyses according to maximal BMI and early age at diagnosis added suggestive evidence of linkage with type 2 diabetes at 7p22-p21 (MLS 3.51), 15q13-q21 (MLS 3.91), and 20q12-q13 (MLS 2.32). These results support previous indication for linkage found on chromosome 3q, 15q, and 20q in other populations and identifies two new potential loci on 7p and 11p that may confer genetic risk for type 2 diabetes in the Japanese population. *Diabetes* 51:1247–1255, 2002

The report of the national survey in 1997 estimated that ~6.9 million people suffer from diabetes in Japan (1). Of these individuals, >90% have type 2 diabetes, and recently, there has been a dramatic increase of type 2 diabetes in Japan, which may be related to both environmental changes and the presence of diabetes-susceptible gene alleles in this population. Indeed, several studies have shown that migration from Japan to the U.S. increases the risk for type 2 diabetes (2), and moreover, people in Japan are increasingly exposed to the Westernized style of living.

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GK, glucokinase; IRS-1, insulin receptor substrate-1; LOD, logarithm of odds; MLS, maximum LOD score; QTL, quantitative trait loci.

It is generally accepted that the development of type 2 diabetes is associated with both insulin secretion defect and insulin resistance. This hypothesis is clearly supported by an animal model–based experiment, in which normoglycemic, insulin-resistant, insulin receptor substrate-1(IRS-1)-null mice and glucokinase $(GK)^{+/-}$ mice with impaired glucose tolerance were crossed. Mice that carry the two mutations ($IRS\text{-}1^{-/-}$ and $GK^{+/-}$) manifest overt diabetes (3). Epidemiological studies demonstrated that in the Japanese population, subjects with a lower insulin response to glucose show a higher risk for later development of type 2 diabetes; that is, insulin secretory defect is a primary metabolic defect in Japanese (4,5). Similar studies in Caucasians showed that impaired insulin sensitivity is the first metabolic defect that predicts the development of type 2 diabetes (6). Thus, Japanese type 2 diabetic individuals might have a different genetic background from other populations that primarily affects the responsiveness of insulin secretion to glucose.

In this regard, molecular screening of Japanese diabetic subjects has shown that the mitochondrial mutation (A to G transition at position 3243, which codes for tRNA-Leu) was present in ~1% of type 2 diabetic individuals in Japan; a much larger prevalence than in Caucasians (7,8). In contrast, mutations in the five mature-onset diabetes of the young (MODY) genes, which are also responsible for primary defects of insulin secretion, were less frequently identified in the Japanese type 2 diabetic patients (1%) than in Caucasians (9–12). Other candidate genes that have been reported to be associated with the genetic risk for type 2 diabetes in Japan include peroxisome proliferator–activated receptor-γ (PPAR-γ) (13), ADRB3 (14,15), uncoupling protein 1 (UCP1), and sulfonylurea receptor (SUR) (16). Supporting data for these genes is rather contradictory and, at this stage, it is likely that most of the genetic factors for type 2 diabetes in the Japanese population may be unknown.

This prompted us to perform a genome-wide scan of Japanese type 2 diabetic families. We show the results of the first whole-genome search of type 2 diabetes susceptibility genes in a Japanese population using 415 microsatellite markers encompassing the whole genomic area (401 markers mainly from ABI linkage mapping set and 14

TABLE 1
Clinical characteristics of 359 Japanese type 2 diabetic subjects

	Men	Women
n	202	157
Age at time of study (years)	59.7 ± 8.6	61.0 ± 9.5
Age at diagnosis (years)	45.9 ± 9.7	48.3 ± 11.1
Current BMI (kg/m^2)	22.7 ± 2.8	23.2 ± 3.6
Maximal BMI (kg/m^2)	26.4 ± 3.0	27.4 ± 3.8

Data are means ± SD, unless otherwise indicated.

supplemental markers for 13 candidate genes expressed in pancreas).

RESEARCH DESIGN AND METHODS

For this study, nuclear Japanese families with at least two siblings with type 2 diabetes were recruited. Families were ascertained through a proband treated for type 2 diabetes, and they were included in the study if the proband had at least one additional sibling with type 2 diabetes. A total of 359 affected individuals (224 affected sib-pairs in 159 families) were available. Diagnosis was based on the World Health Organization's 1985 criteria. Tables 1 and 2 show the clinical characteristics of these subjects. In our population, parents were not available except for one mother in one family.

Previous reports show that in the Japanese population, the frequency of a positive family history of diabetes in Japanese diabetic patients is particularly higher in parents of young-onset type 2 diabetic patients and lower in patients who have maximal BMI >35 kg/m^2 compared with those with maximal BMI <30 kg/m^2 (17). Therefore, we considered BMI and age at onset as possible covariates and/or confounding factors. We designed two subset populations, the first one is the subset termed Young-Onset45, in which both siblings were <45 years of age at diagnosis, and which includes the younger-diagnosed 20% families. The other is the subset termed Lean30, in which both siblings have a maximal BMI <30 kg/m^2 (Table 3).

Genotyping of microsatellite markers. Genomic DNA was isolated from whole blood using the phenol-chloroform method. Genotyping was performed using a fluorescence-labeled human linkage mapping set (PE-LMSV2) comprising 400 highly informative microsatellite markers with an average intermarker spacing of 9.7 cM. Multiplex PCR conditions were set up for each of the 28 panels to amplify the 400 markers in 87 PCRs. PCR (95°C for 12 min, then 40 cycles at 94°C for 15 min, 55°C for 15 min, 72°C for 30 min, and 72°C for 10 min) was performed with a 384-well plate on a GeneAmp PCR system (9700 Biblock; Perkin-Elmer, Foster City, CA) using the following (in 10-μl reactions): 20–40 ng genomic DNA, 2.5 mmol/l $MgCl_2$, 0.25 mmol/l dNTPs (Pharmacia), variable amounts (0.2–1.5 pmol) of 5′ and 3′ primers, and 0.4 units AmpliTaq Gold DNA polymerase (Perkin-Elmer) in 1× PCR buffer II (Perkin-Elmer). (Multiplex PCR conditions are available from the authors on request.) An automated 96-channel pipettor Multimek 96 (Beckman) was used for the pipetting steps. Pooled amplification products were electrophoresed through 5% polyacrylamide gels (Long Ranger Singel Pack; Perkin Elmer) for 1.5 h at 2,000 V on 24-cm plates on an ABI 377 DNA sequencer. Semiautomated fragment sizing was performed by using Genescan 3.0 software (ABI), followed by allele calling with Genotyper 2.1 software (ABI). Some panels were electrophoresed on a multicapillary ABI 3700 sequencer and analyzed by Genescan-2.1 software (Perkin-Elmer). Among 400 markers in PE-LMSV2, eight markers (D1S214, D1S252, D3S2338, D3S1285, D4S1534, D7S640, D15S153, and D19S221) were not included because of technical problems.

In addition to PE-LMSV2, 23 additional markers were genotyped. Also, 13 transcription factors in pancreas were genotyped: D2S1391 (BETA2/NEUROD1); D4S395 (Nkx6.1); D7S504 (PAX4); D11S4154 (PAX6); D12S1349 (hepatocyte nuclear factor-1α [HNF-1α]); D13S221 (IPF-1); D14S75 (HNF-3α); D15S209 (HNF-6); D17S927 and D17S946 (HNF-1β); D19S217 and D19S903 (HNF-3γ); D20S848 (HNF-3β and Nkx2.2); and D20S855 (HNF-4α). For better informativity of some loci, D2S140 (*NIDDM1* locus); D3S3730, D3S3609, D3S3592 and D3S3651 (3q27); D6S305 (6q26); and D11S4098 (11q25) were added. Two markers (D5S429 and D8S1705) were added to fill in 20-cM gaps. Markers D4S403 and D14S68 were replaced by D4S2942 and D14S67, respectively. Finally, a total of 415 markers were genotyped. Genotypes were reviewed independently by two members of the research team to confirm the accuracy of allele calling. The overall dropout rate for the 148,985 genotypes was <4%. The average heterozygosity for the PE-LMSV2 in the Japanese population was 0.72, lower than the 0.79 value found in French Caucasians. Incompatibilities were screened with the PED-CHECK 1.1 program (18). We used the Relative program, which checks for misspecified relationship in families where information is low due to the absence of parents (19).

TABLE 2
Medication history of 359 participants

Medication history	Number of diabetic participants
Diet alone	88 (25)
OHA	181 (50)
INS	88 (25)
OHA+INS	2 (<1)

Data are *n* (%). INS, insulin, OHA, oral hypoglycemic agents.

Nonparametric linkage analyses. Nonparametric two-point and multipoint analyses were performed with the programs Mapmaker and Sibs2.0 (measuring the maximum logarithm of odds score [MLS]) (20), considering all the pairs as independent. Allele frequencies for the parameter file were estimated in our sample through an expectation-maximization algorithm implemented in the FBAT program (21). We also typed five markers in a population of 100 unaffected and unrelated individuals to check the accuracy of the method. No significant difference was observed for any allele frequency estimation. Marker map positions for the genome-wide scan were obtained from the sex-averaged maps compiled by Généthon and are given in Haldane centimorgans (cM). Intermarker distances in our samples were systematically checked with the program Vitesse (22).

To assess the possible confounding effect of BMI (obesity) and age at onset, nonparametric multipoint analyses were performed in two predefined subsets, Young-Onset45 and Lean30. When we applied the criteria of the Young-Onset45 subset in our study population, the sample size was reduced to 36 families and 43 sib-pairs.

Because we conducted these multiple analyses for multiple markers, we completed a simulation study to estimate the genome-wide empirical *P* values. Marker allele frequencies and map distances were kept as in the original sample, and genotypes were dropped through the 159 families using the program Simulate (23), under the hypothesis of no linkage between the disease and the markers. Our aim was to obtain replicates under the same conditions of informativity as the original set: the same allele frequencies and map distances, the same missing individuals for each marker, and the same phenotype for each individual. To estimate the genome-wide *P* values, we simulated 350 replicates with the 22 autosomes each. We then conducted three analyses of each of the replicates for three groups: whole studied population, Young-Onset45 subset, and Lean30 subset. Thus, we completed a total of 1,050 multipoint analyses (350 for each group), and for each replicate we stored the maximum MLS reached (MLS_{max}) and the group-specific maximum MLS (MLS_{whole}, MLS_{YO}, and MLS_{LN} for the whole studied population, Young-Onset45 subset, and Lean30 subset, respectively). We will give two empirical genome-wide *P* values. The first one, $P_{emp\text{-}uncorrected}$, is the probability that MLS_{whole} exceeds an observed MLS. The second one, $P_{emp\text{-}corrected}$, is the probability that MLS_{max} exceeds MLS in the entire experiment: i.e., how many times during 350 analyses the MLS_{max} was greater than the observed MLS. These *P* values account for multiple testing at all positions of the genome, and $P_{emp\text{-}corrected}$ also accounts for stratification according to the phenotype.

TABLE 3
Description of affection status and structure of the 159 nuclear Japanese families

		Affection status for the two subsets	
	Whole population with type 2 diabetes	Young-Onset45 (type 2 diabetes with age at diagnosis <45 years)	Lean30 (type 2 diabetes with maximal BMI <30 kg/m^2)
Total families	159	36	112
Total sib-pairs	224	43	141
Families with			
Two affected sibs	133	34	101
Three affected sibs	23	1	10
Four affected sibs	2	1	0
Five affected sibs	1	0	1

Data are *n*.

We undertook an ordered subset analysis for all chromosomes. In this case, the analyses were conducted using the GenehunterPlus program (24,25). This approach removes some of the arbitrariness that accompanies the subdivision of a sample into subsets. Two covariates, maximal BMI and age at the diagnosis, are included. Families are ranked by the mean value of the covariate in affected siblings, and the cumulative sum of the logarithm of odds (LOD) score grid is evaluated as each family, in rank order, is consecutively added to the analysis. For maximal BMI covariate, the linkage analysis was performed twice, first ranking them from lowest to highest (increasing), and then by ranking the family from highest to lowest (decreasing). The decreasing method enables us to see the possible influence of obesity on diabetes susceptibility. For age at diagnosis, the analysis was performed only once, from lowest to highest family mean (increasing). The MLS and the rank of the family at which the maximum occurs are noted. For each chromosome, we determined an empirical *P* value through random permutation of the family orders simulating 1,000 times. This *P* value is the probability of observing the MLS under the null hypothesis of no effect of the covariate on the linkage magnitude on each chromosome. When BMI is the covariate, the *P* value accounts for the two analyses according to increasing and decreasing classification.

RESULTS

We applied the criteria of Lander and Kruglyak to define regions of significant (LOD 3.6, $P = 0.00002$) or suggestive (LOD 2.2, $P = 0.0007$) linkage in the whole genome. These criteria have been widely adopted and are useful for comparison between publications. Furthermore, we calculated the empirical genome-wide *P* values to quantify the confidence we have in our results. For the evaluation of replication of the linkage established by other studies, we followed the same guidelines indicating that a *P* value <0.01 is required (26).

Two-point results. Among all 415 markers genotyped, 31 markers showed nominal evidence of linkage ($P < 0.05$) in the two-point analysis (Table 4). Moreover, D11S935 in 11p13 demonstrated an MLS of 2.35 ($P = 0.00096$).

Multipoint results. Table 5 summarizes results of the multipoint analyses in the whole studied population. One genetic region, 11p13-p12, demonstrated a multipoint MLS of 3.08 at D11S935 ($P = 0.00017$) and filled the criteria of suggestive linkage at the genome-wide level (Table 5). When analyzing the "whole" sample only, this MLS was reached 25 times in 350 simulated genome scans ($P = 0.07$), and when accounting for the three analyses, it was reached once every five genome scans ($P = 0.2$). Subsequent typing of an additional marker (D11S4154) in this region increased the MLS to 3.32. In addition, seven genetic autosomal intervals, on chromosomes 1p36-p32, 2q34, 3q26-q28, 6p23, 7p22-p21, 15q13-q21, and 20q12-q13, gave an MLS of >1.17, thus showing nominal indication for linkage with type 2 diabetes (Table 5).

When analyzed in the predefined subset populations, Young-Onset45 and Lean30, six intervals (2q34, 7p22-p21, 11p13-p12, 14q11-q13, 15q13-q21, and 20q12-q13) reached the statistical threshold for suggestive linkage (Table 5). By simulating 1,050 genome scans with the three groups (one whole and two subset populations, 350 times for each), we estimated empirical *P* values (in parentheses). An MLS of 2.41 ($P = 0.51$) was found on 2q34 (223.33 cM) in the Lean30 subset, whereas nominal indication of linkage was obtained in the whole population in a 10-cM-apart region (233.87 cM). An MLS of 3.51 ($P = 0.082$) was found on the 7p22-p21 region in the Lean30 subset, whereas nominal linkage in the whole population was found in the same region. An MLS of 3.00 ($P = 0.23$) was found on the 11p13-p12 region in the Lean30 subset, whereas suggestive linkage in the whole population was observed in the same region. In addition, an MLS of 2.37 ($P = 0.52$) was obtained on 14q11-q13 in the Young-Onset45 subset, whereas nominal evidence was not observed in the whole population in this region. Strikingly, the best result was obtained on 15q13-q21, which showed an MLS of 3.91 ($P = 0.034$) in the Young-Onset45 subset and an MLS of 2.44 ($P = 0.5$) in the Lean30 subset, whereas nominal evidence of linkage was found in the whole population. Furthermore on 20q12-q13 a MLS of 2.32 ($P = 0.53$) was found in the Lean30 subset. In this region, nominal evidence of linkage was observed when the whole population was studied.

The ordered subset analyses gave additional support for the interaction of BMI in the 7p22-p21 region, where a MLS of 4.11 was obtained at position 8.12 cM in 81 families, which were increasingly order-ranked by maximal BMI (Table 6). A total of 1,000 simulations gave an empirical *P* value of 0.0080. Moreover, this MLS was observed 120 times on the whole genome for the three ordered subset analyses.

TABLE 4
Results of two-point analyses and markers with nominal evidence of linkage ($P < 0.05$)

Marker	Cytogenetic location	MLS	Nominal *P* value
D1S199	1p36.13	0.81	0.04251
D1S2890	1p32.1	0.94	0.03063
D1S230	1p31.3	1.24	0.01410
D2S2330	2q24.3	0.82	0.04066
D2S325	2q34	1.79	0.00366
D3S1565	3q26.31	1.57	0.00623
D3S3609	3q27	0.79	0.04495
D3S3592	3q27	0.85	0.03776
D3S1580	3q28	1.04	0.02326
D4S406	4q25	0.83	0.04030
D5S428	5q14.3	1.05	0.02290
D7S517	7p22.2	0.79	0.04457
D7S516	7p15.1	0.75	0.04904
D7S630	7q21.13	0.83	0.03978
D9S273	9q13	0.80	0.04304
D10S249	10p15	0.88	0.03489
D11S4154		1.10	0.01998
D11S935	11p13	2.35*	0.00096
D11S905	11p12	1.18	0.01640
D15S994	15q15.1	1.57	0.00631
D15S978	15q21.1	1.34	0.01115
D15S209	15q21.2	0.82	0.04088
D15S117		0.95	0.02923
D16S423	16p13.3	0.85	0.03841
D16S520	16q24.1	0.84	0.03908
D19S226		1.19	0.01613
D19S903		0.82	0.04077
D20S889	20p13	1.14	0.01830
D20S112	20p11.23	1.30	0.01212
D20S855	20q12	1.34	0.01098
D20S119	20q12	1.14	0.01820

*Supporting suggestive linkage (LOD >2.2, $P < 0.0007$) in genome-wide significance.

DISCUSSION

The presented genome scan in a Japanese population showed that the 11p13-p12 locus gave suggestive linkage with type 2 diabetes and that a possible implication exists

TABLE 5
Results of multipoint analyses showing loci with nominal evidence of linkage (MLS >1.17) in the whole population and two predefined subsets their empirical *P* values

Cytogenetic location	Calculated peak position (cM)	Adjacent marker	Subset	MLS	Nominal *P* value	$P_{emp\text{-}uncorrected}$ (1 trait)	$P_{emp\text{-}corrected}$ (3 traits)
1p36-p32	57.13	D1S199	Whole	1.53	0.00699	0.76	0.99
2q34	233.87	D2S325	Whole	1.68	0.00470	0.66	0.96
3q26-q28	216.73	D3S1565	Whole	1.38	0.01002	0.86	0.99
6p23	23.75	D6S289	Whole	1.52	0.00718	0.76	0.99
7p22-p21	10.29	D7S517	Whole	1.80	0.00354	0.58	0.91
11p13-p12	54.23	D11S935	Whole	3.08*	0.00017	0.07	0.20
15q13-q21	42.75	D15S994	Whole	2.19	0.00138	0.32	0.70
20q12-q13	66.15	D20S119	Whole	1.67	0.00489	0.66	0.96
1p36-p32	85.55	D1S2797	Lean30	1.58	0.00617	—	0.97
2q34	223.33	D2S117	Lean30	2.41*	0.00082	—	0.51
2q36	261.76	D2S396	Young-Onset45	1.68	0.00473	—	0.96
3p14	110.48	D3S1566	Young-Onset45	1.73	0.00426	—	0.95
4q31	201.34	D4S415	Young-Onset45	1.63	0.00547	—	0.97
7p22-p21	10.29	D7S517	Lean30	3.51*	0.00006	—	0.082
11p13-p12	54.23	D11S935	Lean30	3.00*	0.00020	—	0.23
14q11-q13	24.51	D14S275	Young-Onset45	2.37*	0.00090	—	0.52
15q13-q21	42.36	D15S994	Young-Onset45	3.91†	0.00002	—	0.034
15q13-q21	42.36	D15S994	Lean30	2.44*	0.00076	—	0.5
18q23	135.66	D18S70	Young-Onset45	1.52	0.00715	—	0.98
19q13	93.26	D19S571	Young-Onset45	1.69	0.00466	—	0.96
20q12-q13	66.15	D20S119	Lean30	2.32*	0.00102	—	0.53

*Supporting suggestive linkage (LOD >2.2, $P < 0.0007$) according to genome-wide criteria; †supporting significant linkage (LOD > 3.6, $P < 0.00002$) according to genome-wide criteria.

for seven loci on chromosomes 1, 2, 3, 6, 7, 15, and 20, which show nominal evidence for linkage (MLS >1.17).

At the 11p13-p12 locus, multipoint analysis showed an MLS of 3.08 ($P = 0.00017$) at 54.23 cM in our genetic map (Fig. 1). This multipoint result is supported by two-point results of three markers: D11S4154 (nominal $P = 0.01998$), D11S935 (nominal $P = 0.00096$), and D11S905 (nominal $P = 0.01640$) (Table 4). Moreover, the fact that the LOD score increased to 3.32 when we typed an additional marker is encouraging. One transcription factor gene, PAX6, is located between D11S4154 and D11S935. Pax6 is required for normal islet development and is a key transcriptional regulator of the pancreatic α-cell hormone glucagon (27). No evidence for linkage was found in other genome scans. Extension of the study with additional sib-pairs is indispensable to definitely claim the linkage in this region.

On 7p22-p21, the nominal evidence of linkage group is enhanced in leaner sib-pairs compared with the whole-group analysis (Fig. 2). An MLS of 3.51 was found on the 7p22-p21 region in the Lean30 subset. To avoid the arbitrariness of dividing the subjects into a group by defined BMI <30 kg/m^2, we performed ordered subset analysis with covariate maximal BMI and increased-ordered rank, where families are ordered by the average of maximal BMI of sib-pairs and ranked from lowest to highest. As presented in Table 6, a MLS of 4.11 was observed in the ordered subset analysis, including 81 families with low mean BMI. The chromosome-wide empirical *P* value obtained with 1,000 simulations was 0.0080. This result supports the idea that the linkage could be modulated by the degree of obesity in the sib-pairs. In the Finnish genome scan, a quantitative trait loci (QTL) for fasting glucose level in nondiabetic subjects was reported on 7p at position 0.0 cM, showing a modest MLS of 1.24 (28). No other type 2 diabetes genome scan identified this region

TABLE 6
Results of ordered subset analysis (MLS >2.0)

Chromosome	Calculated peak position (cM)	Adjacent marker	Covariate	Rank order	Number of families	Max LOD score	Empirical *P* value
1	53.26	D1S199	Max BMI	Increasing	70	2.14	0.2800
3	115.65	D3S1566	Max BMI	Increasing	53	2.42	0.1060
6	34.32	D6S289	Max BMI	Decreasing	69	2.67	0.0460
7	8.12	D7S517	Max BMI	Increasing	81	4.11	0.0080
7	10.29	D7S517	Age at diagnosis	Increasing	135	2.62	0.2980
9	72.60	D9S175	Max BMI	Increasing	95	2.40	0.1080
11	54.23	D11S935	Max BMI	Increasing	130	2.91	0.2720
11	54.23	D11S935	Age at diagnosis	Increasing	73	2.46	0.4720
15	42.36	D15S994	Age at diagnosis	Increasing	107	2.16	0.1020
19	60.37	D19S414	Age at diagnosis	Increasing	88	2.53	0.1200

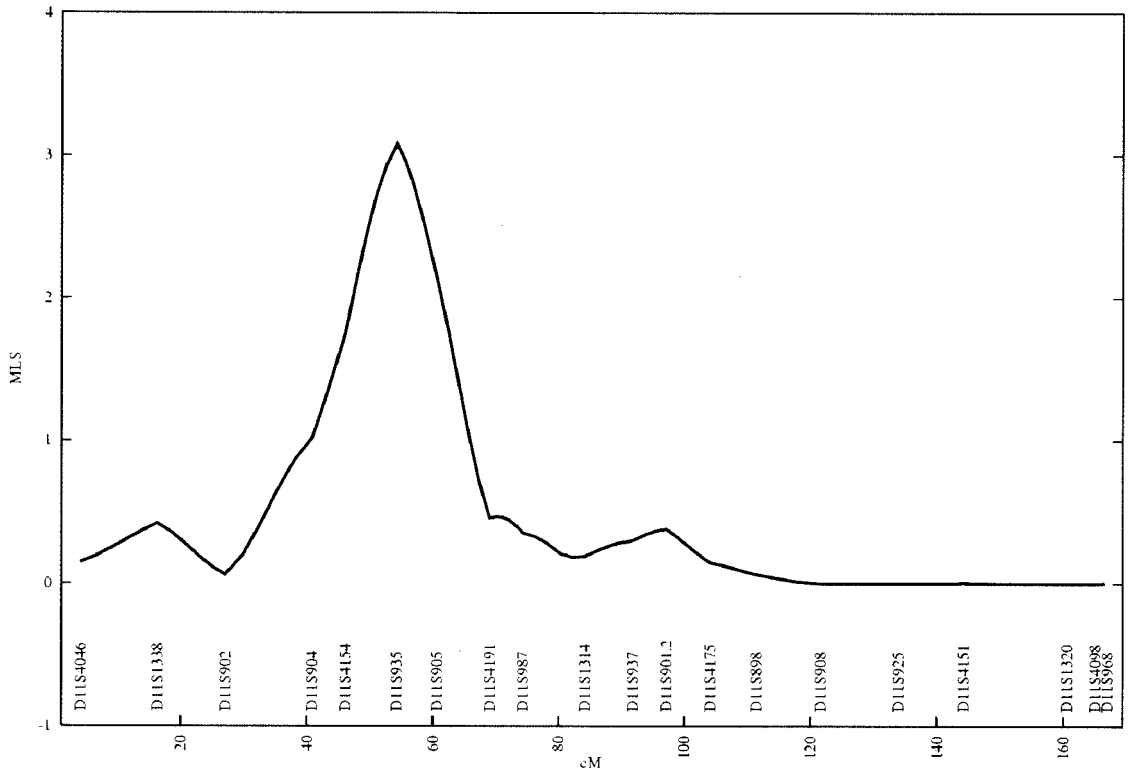

FIG. 1. Results of MLS multipoint analyses for chromosome 11. The horizontal axis corresponds to the genetic map, and the vertical axis indicates MLS. The curve corresponds to the whole studied population.

so far, and no obvious candidate genes are known, although the gene for protein kinase A regulatory Iβ subunit (PRKAR1B) is mapped on 7p22.

Other evidence for linkage was provided by subset analyses on chromosome 15q13-q21. Stratification according to the age of onset gave significant evidence for linkage on 15q13-q15 (MLS 3.91, $P = 0.00002$) in 36 families in which both sib-pairs developed type 2 diabetes before the

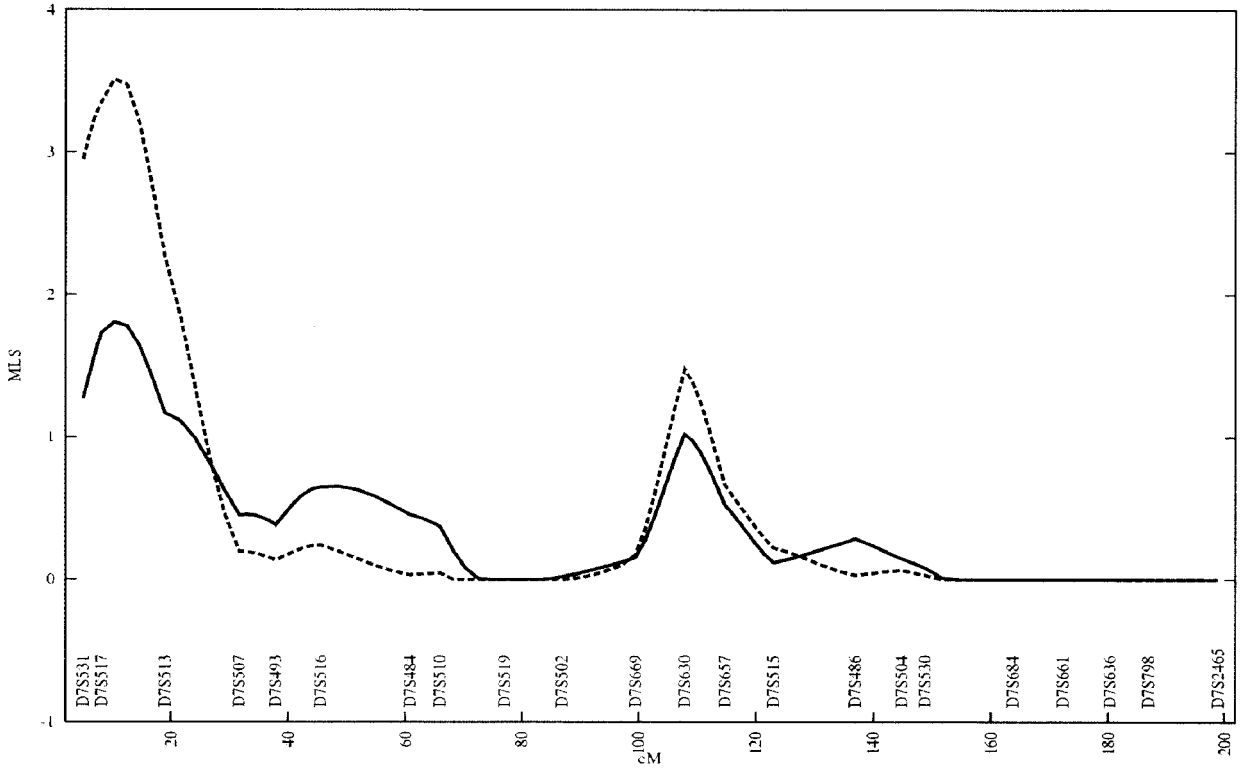

FIG. 2. Results of MLS multipoint analyses for chromosome 7. The horizontal axis corresponds to the genetic map, and the vertical axis indicates MLS. The curves with the solid line corresponds to the whole studied population, and the curve with the broken line corresponds to the Lean30 subset.

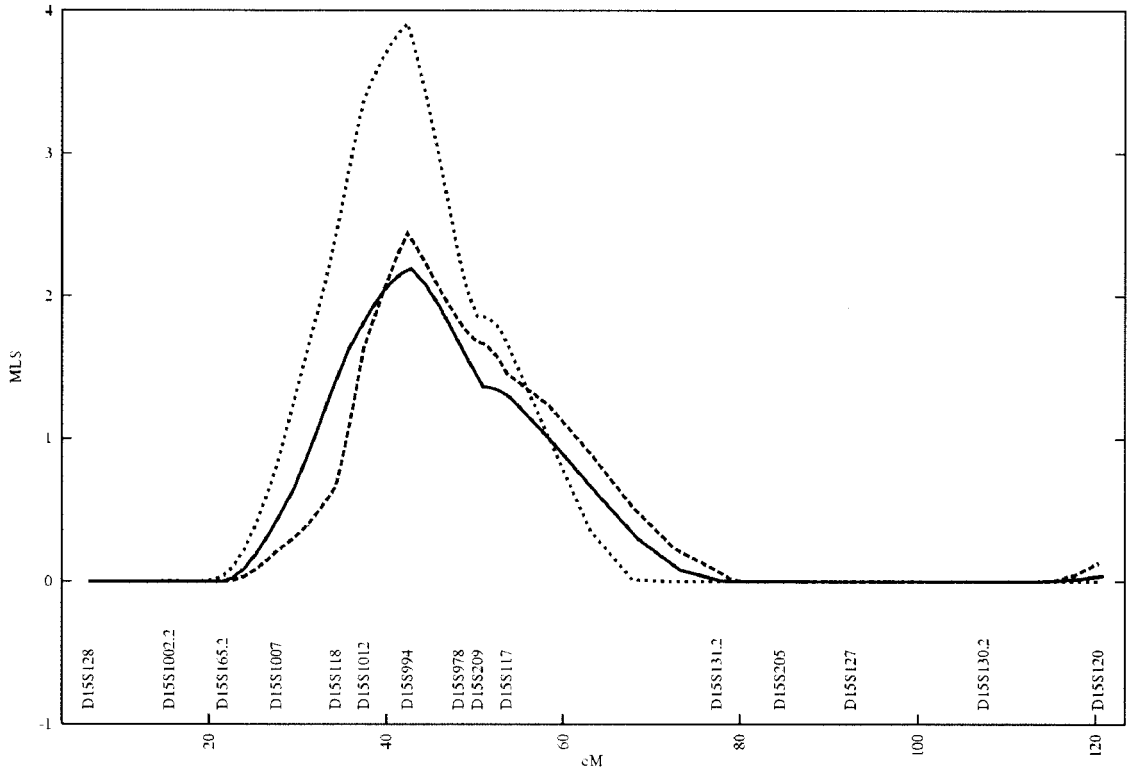

FIG. 3. Results of MLS multipoint analyses for chromosome 15. The horizontal axis corresponds to the genetic map, and the vertical axis indicates MLS. The curves with solid, broken, and dotted lines correspond to the whole studied population, the Lean30 subset, and the Young-Onset45 subset, respectively.

age of 45 years. We believe that this chromosome 15q linkage might be true for several reasons. First, the linkage, although not significant in the whole Japanese cohort (MLS 2.19, $P = 0.00138$), filled the criteria for the replication of the previously established region in Mexican-Americans. Moreover, Cox et al. (29) showed that in Mexican-Americans, this chromosome 15q locus was in close interaction with the niddm1/CAPN10 locus on chromosome 2q. Second, the indication for linkage found in the whole Japanese cohort is reinforced in both lean and young-onset subsets (Fig. 3). In addition, these two conditions seem to be associated with a higher genetic risk for type 2 diabetes within sib-ships (Warram and colleagues, unpublished data). Finally, the empirical P value of MLS = 3.91, indicating the probability of observing a higher MLS by chance anywhere in the genome in any of the three analyses (one whole and two subset populations), was 0.034. Interestingly, the calpain-3 gene (CAPN3) is situated between D15S994 and D15S978, the markers that supported the linkage in Japanese families. Therefore, CAPN3 appears to be a major positional candidate gene for Japanese type 2 diabetes susceptibility genes.

For the chromosome 20q12-q13 locus, the first indication of linkage with type 2 diabetes in the whole population became suggestive when lean sib-pairs were analyzed (MLS 2.32) (Fig. 4). Importantly, several studies already pointed out the existence of type 2 diabetes susceptibility gene(s) in this region (30–34). We have previously reported evidence of linkage with type 2 diabetes in French diabetic families on 20q, especially in large multigenerational pedigrees (30) and in young-age-of-onset sib-pairs (31). Moreover, Ghosh and colleagues reported their best genome scan results in Finnish populations for this region, with an MLS of 2.15 (32,33). One of the obvious candidate genes in this region is HNF-4α. Two-point results of adjacent markers D20S885 and D20S119 are compatible with a possible role of this gene or of an unknown gene located nearby. Interestingly, one Japanese study showed that one single nucleotide polymorphism in intron1b (−5 C/T substitution) in the HNF-4α gene may be associated with type 2 diabetes in this population (35). Further linkage disequilibrium studies in this region will help to address this issue.

Our group recently reported in French type 2 diabetes families a linkage on 1q21-q24 that corresponds to a locus found by several other groups (36–38). In the present Japanese study, we found a small peak in the near region (at D1S196, with an MLS of 0.79), which was not sufficient for replication. In the French genome scan, the best result was seen on 3q27-qter (38). These data were replicated in White Americans, where several significant QTLs associated with metabolic traits (BMI, leptin, insulin, and anthropometric measurements) were shown in the same region (39). Our Japanese genome scan confirms these findings. A modest multipoint MLS of 1.38 ($P = 0.01$) was found on 3q26-q28 in the whole population, and two-point results of four markers (D3S1565, D3S3609, D3S3592, and D3S1580) in this region had nominal evidence of linkage ($P < 0.05$). This region presents putative candidate genes, including GLUT2 (SLC2A2), apolipoprotein D (APOD), phosphatidylinositol 3-kinase catalytic p110α subunit (PI3KCA), and adiponectin (APM1 or GBP28). Two studies have reported a polymorphism in the apolipoprotein D locus to be associated with diabetes and obesity (40,41). We recently observed that polymorphisms in the APM1/adiponectin gene were associated with type 2 diabetes in both the

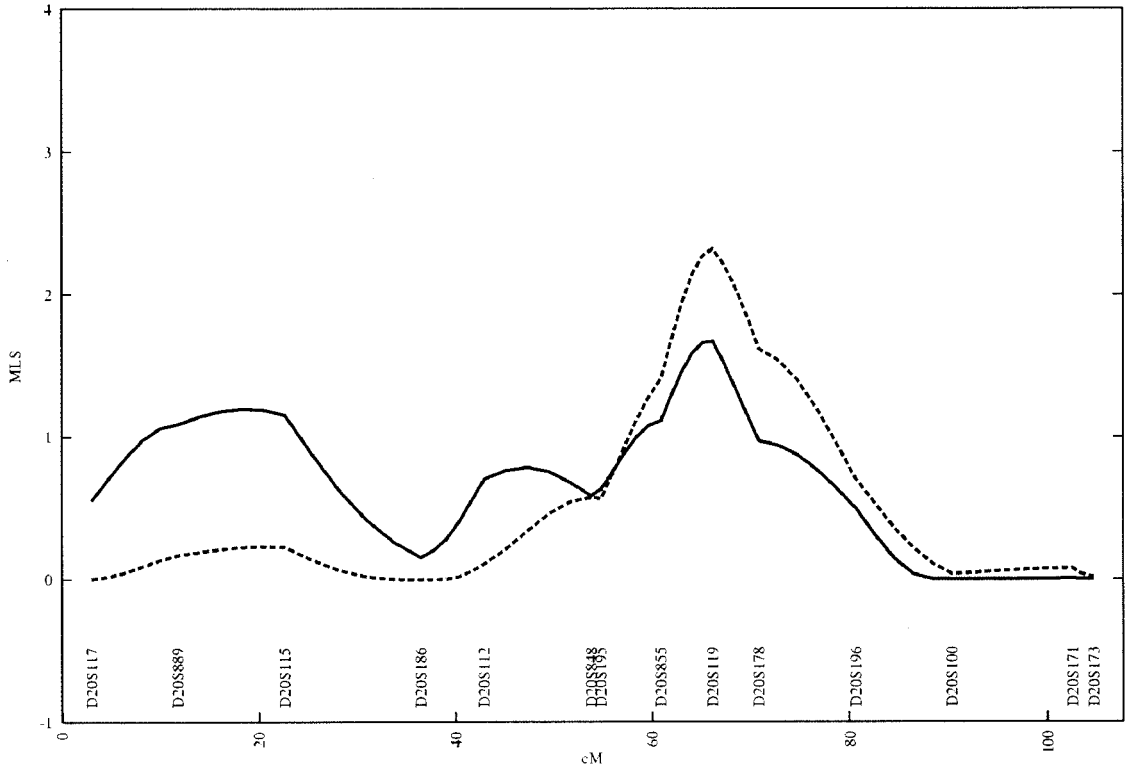

FIG. 4. Results of MLS multipoint analyses for chromosome 20. The horizontal axis corresponds to the genetic map, and the vertical axis indicates MLS. The curves with solid and broken lines correspond to the whole studied population and the Lean30 subset, respectively.

Japanese and French populations. Additional single nucleotide polymorphism analyses will be necessary to determine whether this gene contributes to the observed linkage.

Besides the five region discussed above, 1p36-p32, 2q34, and 6p23 showed an MLS >1.17, which is sufficient for replication, but no confirmed linkage has been reported in these regions. Recently, two loci on 9p were reported to be linked to type 2 diabetes in a Chinese population (42). The D9S171 and D9S175 loci showed suggestive evidence of linkage (nonparametric linkage value of 3.286 and 2.939, respectively). In our Japanese study, in ordered subsets, we also have a MLS of 2.40 at D9S175 in the 95 families with lower maximum BMI (Table 6). Another locus in Chinese samples was on 20q, corresponding to D20S196, which is 14 cM from D20S119. These results may suggest the existence of common type 2 diabetes susceptibility loci between Chinese and Japanese populations.

Although our results in some respect overlap with others in regard to 3q, 15q, and 20q, we observed no linkage in 2q37 (29,43), 10q (44), 11q23-q25 (36), 12q24 (45), or 18p11 (46). Considering our limited sample size (224 sib-pairs), this failure of replication may be explained by different ethnic backgrounds or our limited power to detect linkage with genes having marginal effects in the Japanese population. On the other hand, two regions have been newly identified in this study, the first ones in the Japanese population, on 7p22-p21 and on 11p13-p12. Although we couldn't exclude that one or more locus is a false positive result, fine mapping of these regions and replication in other family samples of Japanese origin will address this issue. In addition, these findings will provide evidence for the investigation of positional candidate genes in the Japanese type 2 diabetes population.

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REFERENCES

1. Office for Lifestyle-Related Disease Control, Ministry of Health and Welfare: *Diabetes Survey 1997*. Tokyo, Ministry of Health and Welfare, Government of Japan, 1999
2. Fujimoto WY, Leonetti DL, Kinyoun JL, Newell-Morris L, Shuman WP, Stolov WC, Wahl PW: Prevalence of diabetes mellitus and impaired glucose tolerance among second-generation Japanese-American men. *Diabetes* 36:721–729, 1987
3. Terauchi Y, Iwamoto K, Tamemoto H, Komeda K, Ishii C, Kanazawa Y, Asanuma N, Aizawa T, Akanuma Y, Yasuda K, Kodama T, Tobe K, Yazaki Y, Kadowaki T: Development of non-insulin-dependent diabetes mellitus in the double knockout mice with disruption of insulin receptor substrate-1 and beta cell glucokinase genes: genetic reconstitution of diabetes as a polygenic disease. *J Clin Invest* 99:861–866, 1997
4. Kosaka K, Hagura R, Kuzuya T: Insulin responses in equivocal and definite diabetes, with special reference to subjects who had mild glucose intolerance but later developed definite diabetes. *Diabetes* 26:944–952, 1977
5. Kadowaki T, Miyake Y, Hagura R, Akanuma Y, Kajinuma H, Kuzuya N, Takaku F, Kosaka K: Risk factors for worsening to diabetes in subjects with impaired glucose tolerance. *Diabetologia* 26:44–49, 1984
6. Martin BC, Warram JH, Krolewski AS, Bergman RN, Soeldner JS, Kahn CR: Role of glucose and insulin resistance in development of type 2 diabetes mellitus: results of a 25-year follow-up study. *Lancet* 340:925–929, 1992
7. Kadowaki T, Kadowaki H, Mori Y, Tobe K, Sakuta R, Suzuki Y, Tanabe Y, Sakura H, Awata T, Goto Y, et al.: A subtype of diabetes mellitus associated with a mutation of mitochondrial DNA. *N Engl J Med* 330:962–968, 1994
8. Otabe S, Sakura H, Shimokawa K, Mori Y, Kadowaki H, Yasuda K, Nonaka K, Hagura R, Akanuma Y, Yazaki Y, Kadowaki T: The high prevalence of diabetic patients with a mutation in the mitochondrial gene in Japan. *J Clin Endocrinol Metab* 79:768–771, 1994
9. Sakura H, Eto K, Kadowaki H, Shimokawa K, Ueno H, Koda N, Fukushima Y, Akanuma Y, Yazaki Y, Kadowaki T: Structure of the human glucokinase

gene and identification of a missense mutation in a Japanese patient with early-onset non-insulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 75:1571–1573, 1992
10. Eto K, Sakura H, Shimokawa K, Kadowaki H, Hagura R, Akanuma Y, Yazaki Y, Kadowaki T: Sequence variations of the glucokinase gene in Japanese subjects with NIDDM. *Diabetes* 42:1133–1137, 1993
11. Iwasaki N, Oda N, Ogata M, Hara M, Hinokio Y, Oda Y, Yamagata K, Kanematsu S, Ohgawara H, Omori Y, Bell GI: Mutations in the hepatocyte nuclear factor-1alpha/MODY3 gene in Japanese subjects with early- and late-onset NIDDM. *Diabetes* 46:1504–1508, 1997
12. Furuta H, Iwasaki N, Oda N, Hinokio Y, Horikawa Y, Yamagata K, Yano N, Sugahiro J, Ogata M, Ohgawara H, Omori Y, Iwamoto Y, Bell GI: Organization and partial sequence of the hepatocyte nuclear factor-4 alpha/MODY1 gene and identification of a missense mutation, R127W, in a Japanese family with MODY. *Diabetes* 46:1652–1657, 1997
13. Hara K, Okada T, Tobe K, Yasuda K, Mori Y, Kadowaki H, Hagura R, Akanuma Y, Kimura S, Ito C, Kadowaki T: The Pro12Ala polymorphism in PPAR: gamma2 may confer resistance to type 2 diabetes. *Biochem Biophys Res Commun* 271:212–216, 2000
14. Sakane N, Yoshida T, Umekawa T, Kogure A, Takakura Y, Kondo M: Effects of Trp64Arg mutation in the beta 3-adrenergic receptor gene on weight loss, body fat distribution, glycemic control, and insulin resistance in obese type 2 diabetic patients. *Diabetes Care* 20:1887–1890, 1997
15. Kadowaki H, Yasuda K, Iwamoto K, Otabe S, Shimokawa K, Silver K, Walston J, Yoshinaga H, Kosaka K, Yamada N, et al.: A mutation in the beta 3-adrenergic receptor gene is associated with obesity and hyperinsulinemia in Japanese subjects. *Biochem Biophys Res Commun* 215:555–560, 1995
16. Ohta Y, Tanizawa Y, Inoue H, Hosaka T, Ueda K, Matsutani A, Repunte VP, Yamada M, Kurachi Y, Bryan J, Aguilar-Bryan L, Permutt MA, Oka Y: Identification and functional analysis of sulfonylurea receptor 1 variants in Japanese patients with NIDDM. *Diabetes* 47:476–481, 1998
17. Hagura R, Matsuda A, Kuzuya T, Yoshinaga H, Kosaka K: Family history of diabetic patients in Japan. *Diabetes Res Clin Pract* 24 (Suppl.):S69–S73, 1994
18. O'Connell JR: PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet* 63:259–266, 1998
19. Göring HH, Ott J: Relationship estimation in affected sib pair analysis of late-onset disease. *Eur J Hum Genet* 5:69–77, 1997
20. Kruglyak L, Lander ES: Complete multipoint sib-pair analysis of qualitative and quantitative traits. *Am J Hum Genet* 57:439–454, 1995
21. Horvath S, Xu X, Laird N: The family based association test method: strategies for studying general genotype-phenotype associations. *Euro J Hum Gen* 9:301–306, 2001
22. O'Connell JR, Weeks DE: The VITESSE algorithm for rapid exact multilocus linkage analysis via genotype set-recoding and fuzzy inheritance. *Nat Genet* 62:659–668, 1995
23. Terwilliger JD, Speer M, Ott J: Chromosome-based method for rapid computer simulation in human genetic linkage analysis. *Genet Epidemiol* 10:217–224, 1993
24. Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES: Parametric and nonparametric linkage analysis: a unified multipoint approach. *Am J Hum Genet* 58:1347–1363, 1996
25. Cox NJ, Frigge M, Nicolae DL, Concannon P, Hanis CL, Bell GI, Kong A: Loci on chromosomes 2 (NIDDM1) and 15 interact to increase susceptibility to diabetes in Mexican Americans. *Nat Genet* 21:213–215, 1999
26. Lander E, Kruglyak L: Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet* 11:241–247, 1995
27. Sander M, Neubuse A, Kalamaras J, Ee HC, Martin GR, German MS: Genetic analysis reveals that PAX6 is required for normal transcription of pancreatic hormone genes and islet development. *Genes Dev* 11:1662–1673, 1997
28. Watanabe RM, Ghosh S, Langefeld CD, Valle TT, Hauser ER, Magnuson VL, Mohlke KL, Silander K, Ally DS, Chines P, Blaschak-Harvan J, Douglas JA, Duren WL, Epstein MP, Fingerlin TE, Kaleta HS, Lange EM, Li C, McEachin RC, Stringham HM, Trager E, White PP, Balow J Jr, Birznieks G, Chang J, Eldridge W, Erdos MR, Karanjawala E, Knapp JI, Kudelko K, Martin C, Morales-Mena A, Musick A, Musick T, Pfahl C, Porter R, Rayman JB, Rha D, Segal L, Shapiro S, Sharaf R, Shurtleff B, So A, Tannenbaum J, Te C, Tovar J, Unni A, Welch C, Whiten R, Witt A, Kohtamäki K, Ehnholm C, Eriksson J, Toivanen L, Vidgren G, Nylund SJ, Tuomilehto-Wolf E, Ross EH, Demirchyan E, Hagopian WA, Buchanan TA, Tuomilehto J, Bergman RN, Collins FS, Boehnke M: The Finland-United States investigation of non-insulin-dependent diabetes mellitus genetics (FUSION) study. II. An autosomal genome scan for diabetes-related quantitative-trait loci. *Am J Hum Genet* 67:1186–1200, 2000
29. Hanis CL, Boerwinkle E, Chakraborty R, Ellsworth DL, Concannon P, Stirling B, Morrison VA, Wapelhorst B, Spielman RS, Gogolin-Ewens KJ, Shephard JM, Williams SR, Risch N, Hinds D, Iwasaki N, Ogata M, Omori Y, Petzold C, Rietzch H, Schröder H-E, Schulze J, Cox NJ, Menzel S, Boriraj VV, Chen X, Lim LR, Lindner T, Mereu LE, Wang Y-Q, Xiang K, Yamagata K, Yang Y, Bell GI: A genome-wide search for human non-insulin-dependent (type 2) diabetes genes reveals a major susceptibility locus on chromosome 2. *Nat Genet* 13:161–166, 1996
30. Hani EH, Zouali H, Philippi A, Beaudoin JC, Vionnet N, Passa P, Demenais F, Froguel P: Indication for genetic linkage of the phosphoenolpyruvate carboxylase (PCK1) gene region on chromosome 20q to non insulin dependent diabetes mellitus. *Diabetes Metab* 22:451–454, 1996
31. Zouali H, Hani EH, Philippi A, Vionnet N, Beckmann J, Demenais F, Froguel P: A susceptibility locus for early-onset non-insulin-dependent (type 2) diabetes mellitus maps to chromosome 20q, proximal to the phosphoenolpyruvate carboxykinase gene. *Hum Mol Gen* 6:1401–1408, 1997
32. Ghosh S, Watanabe RM, Hauser ER, Valle TT, Magnuson VL, Erdos MR, Langefeld CD, Balow J Jr., Ally DS, Kohtamäki K, Chines P, Birznieks G, Keleta HS, Musick ATC, Tannenbaum J, Eldridge W, Shapiro S, Martin C, Witt A, So A, Chang J, Shurtleff B, Porter R, Kudelko K, Unni A, Segal L, Sharaf R, Blaschak-Harvan J, Eriksson J, Tenkula T, Vidgren G, Ehnholm C, Tuomilehto-Wolf E, Hagopian W, Buchanan TA, Tuomilehto J, Bergman RN, Collins FS, Boehnke M: Type 2 diabetes: evidence for linkage on chromosome 20 in 716 Finnish affected sib pairs. *Proc Natl Acad Sci U S A* 96:2198–2203, 1999
33. Ghosh S, Watanabe RM, Valle TT, Hauser ER, Magnuson VL, Langefeld CD, Ally DS, Mohlke KL, Silander K, Kohtamäki K, Chines P, Balow J Jr, Birznieks G, Chang J, Eldridge W, Erdos MR, Karanjawala E, Knapp JI, Kudelko K, Martin C, Morales-Mena A, Musick A, Musick T, Pfahl C, Porter R, Rayman JB, Rha D, Segal L, Shapiro S, Sharaf R, Shurtleff B, So A, Joyce T, Tannenbaum J, Te C, Tovar J, Unni A, Welch C, Whiten R, Witt A, Blaschak-Harvan J, Douglas JA, Duren WL, Epstein MP, Fingerlin TE, Kaleta HS, Lange EM, Li C, McEachin RC, Stringham HM, Trager E, White PP, Eriksson J, Toivanen L, Vidgren G, Nylund SJ, Tuomilehto-Wolf E, Ross EH, Demirchyan E, Hagopian WA, Buchanan TA, Tuomilehto J, Bergman RN, Collins FS, Boehnke M: The Finland-United States investigation of non-insulin-dependent diabetes mellitus genetics (FUSION) study. I. An autosomal genome scan for genes that predispose to type 2 diabetes. *Am J Hum Genet* 67:1174–1185, 2000
34. Klupa T, Malecki MT, Pezzolesi M, Ji L, Curtis S, Langefeld CD, Rich SS, Warram JH, Krowlewski AS: Further evidence for a susceptibility locus for type 2 diabetes on chromosome 20q13.1-q13.2. *Diabetes* 49:2212–2216, 2000
35. Sakurai K, Seki N, Fujii R, Yagui K, Tokunaga Y, Shimada F, Makino H, Suzuki Y, Hashimoto N, Saito Y, Egashira T, Matsui K, Konatsuka A: Mutations in the hepatocyte nuclear factor-4alpha gene in Japanese with non-insulin-dependent diabetes: a nucleotide substitution in the polypyrimidine tract of intron 1b. *Horm Metab Res* 32:316–320, 2000
36. Hanson RL, Ehm MG, Pettitt DJ, Prochazka M, Thompson DB, Timberlake D, Foroud T, Kobes S, Baier L, Burns DK, Almasy L, Blangero J, Garvey WT, Bennet PH, Knowler WC: An autosomal genomic scan for loci linked to type II diabetes mellitus and body-mass index in Pima Indians. *Am J Hum Genet* 63:1130–1138, 1998
37. Elbein SC, Hoffman MD, Teng K, Leppert MF, Hasstedt SJ: A genome-wide search for type 2 diabetes susceptibility genes in Utah Caucasians. *Diabetes* 48:1175–182, 1999
38. Vionnet N, Hani EH, Dupont S, Gallina S, Francke S, Dotte S, De Matos F, Durand E, Leprêtre F, Lecoeur C, Gallina P, Zekiri L, Dina C, Froguel P: Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early-onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21–q24. *Am J Hum Genet* 67:1470–480, 2000
39. Kissebah AH, Sonnenberg GE, Myklebust J, Goldstein M, Broman K, James RG, Marks JA, Krakower GR, Jacob HJ, Weber J, Martin L, Blangero J, Comuzzie AG: Quantitative trait loci on chromosome 3 and 17 influence phenotypes of the metabolic syndrome. *Proc Natl Acad Sci U S A* 97:14478–14483, 2000
40. Hitman GA, McCarthy MI, Mohan V, Viswananthan M: The genetics of non-insulin-dependent diabetes mellitus in South India: an over view. *Ann Med* 24:491–497, 1992
41. Vijayaraghavan S, Hitman GA, Kopelman PG: Apolipoprotein-D polymorphism: a genetic marker for obesity and hyperinsulinemia. *J Clin Endocrinol Metab* 79:568–570, 1994
42. Luo TH, Zhao Y, Li G, Yuan WT, Zhao JL, Huang W, Luo M: A genome-wide search for type II diabetes susceptibility genes in Chinese Hans. *Diabetologia* 44:501–506, 2001

43. Horikawa Y, Oda N, Cox NJ, Li X, Orho-Melander M, Hara M, Hinokio Y, Linder TH, Mashima H, Schwartz PEH, Bosque-Plata L, Horikawa Y, Oda Y, Yoshiuchi I, Colilla S, Polonsky KS, Wei S, Concannon P, Iwasaki N, Schulze J, Baier LJ, Bogardus C, Groop L, Boerwinkle E, Hanis CL, Bell GI: Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat Genet* 26:163–175, 2000
44. Duggirala R, Blangero J, Almasy L, Dyer TD, Williams KL, Leach RJ, O'Connell, Stern MP: Linkage of type 2 diabetes mellitus and of age at onset to a genetic location on chromosome 10q in Mexican Americans. *Am J Hum Genet* 64:1127–1140, 1999
45. Mahtani MM, Widen E, Lehto M, Thomas J, McCarth M, Brayer J, Bryant B, Chan G, Daly M, Forsblom C, Kannine T, Kirby A, Kruglyak L, Munnelly K, Parkkonen M, Reve-Daly MP, Weaver A, Brettin T, Duyk G, Lander ES, Groop LC: Mapping of a gene for type 2 diabetes associated with an insulin secretion defect by a genome scan in Finnish families. *Nat Genet* 14:90–94, 1996
46. Parker A, Meyer J, Lewitzky S, Rennich JS, Chan G, Thomas JD, Orho-Melander M, Lehtovirta M, Forsblom C, Hyrkkö A, Carlsson M, Lindgren C, Groop LC: A gene conferring susceptibility to type 2 diabetes in conjunction with obesity is located on chromosome 18p11. *Diabetes* 50:675–680, 2001