NOVEL GENETIC FINDINGS IN A CHINESE FAMILY WITH EARLY-ONSET FEMALE-RELATED TYPE 2 DIABETES

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Abstract

No inheritance of early-onset female-related type 2 diabetes was reported within Chinese families. In this study, we aim to describe the inheritance pattern of type 2 diabetes in a 3-generation family and identify the gene responsible for type 2 diabetes. Genome-wide multipoint parametric linkage analysis revealed a maximum multipoint logarithm of odds (lod) score of 2.1 for a locus being associated with type 2 diabetes in this family on chromosome 20p11.2-12 between 23.5~30.8cM. Type 2 diabetes may be transmitted as an autosomal dominant trait with a high female-related penetrance in this family. Here we describe the first genetic locus for type 2 diabetes at chromosome 20p11.2-12. This region contains 8 known or predicted genes (PLCB1, PLCB4, LAMP5, PAK7, ANKEF1, SNAP25, SLX4IP, and JAG1). Gene SNAP25 which linked to energy or glucose homeostasis associated phenotypes may play a role in the development of type 2 diabetes in this family.

Key words: type 2 diabetes family, chromosome 20p11.2-12, linkage analysis.

INTRODUCTION

Type 2 diabetes mellitus (T2DM) has become a major public health concern due to its increasing worldwide prevalence over the past three decades (1, 2). T2DM is a metabolic disorder of increasing insensitivity to the action of circulating insulin on its target tissues or of impaired insulin secretion (3). Development of T2DM is the result of interactions between multiple genetic and environmental factors, thus, even with the same environmental exposures, some people are more susceptible to developing T2DM than others, indicating that genetic risk plays a clear role in the development of diabetes (4).

Previous studies have suggested a genetic

basis for T2DM. For example, first degree relatives of individuals with diabetes mellitus (DM) are about 3 times more likely to develop the disease than individuals without a positive family history (5). Therefore, family-based studies, based on individuals with the same genetic background, are a promising method for identifying rare disease variants (6, 7). To date, many T2DM susceptibility loci have been identified through linkage-analysis studies of families (e.g., 2q37 (8), 15q21(9), 10q26 (10), 3p (11), 9p24 (12) in Mexican Americans; 12q24 (13), 1q21-1q23 (14), 9p23 (15) in Caucasians; 1q21-1q23, 11q23-q25 (16), 9p24.1 (17) in Pima Indians; 9p21.3(18), 6q21-q23 and 1q21-q24 (19) in Chinese; and 5q34-q35.2 (20) in Icelanders) that confer genetic risk for this metabolic disorder. The large number of identified loci underscores the genetic heterogeneity for type 2 diabetes between diverse ethnic groups. Here, to better understand the genetics of T2DM within Chinese populations we examined a Chinese family that shows female-related inheritance of type 2 diabetes with a high level of morbidity (50%). We hypothesized that these family members have both similar genetic backgrounds for T2DM and share similar environmental exposures.

MATERIALS AND METHODS

Informed consent conforming to the tenets of the Declaration of Helsinki and following the guidance of sample collection of Human Genetic Disease (863 program) by the Ministry of Public Health of China were obtained from each participant prior to the study. Diagnosis and classification of diabetes was based on the guidelines of the Expert Committee Report of the American Diabetes Association (21). Genomic DNA from 13 members of a Chinese family consisting of 7

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Figure 1. Pedigree of the three-generation family 1 with type 2 diabetes. All family members were used for the linkage analysis. Affected individuals were marked by filled black symbols. Gray symbols indicate unknown phenotype. The proband was marked by an arrow.

Figure 2. Linkage of T2DM in family 1 to chromosome 20p11.2-12. Genome-wide parametric multipoint lod score profile for T2DM family 1 based on SNP data generated with the Human Omni ZhongHua-8 Bead Chip array. Chromosome 20 is displayed from its p-terminal (left) to q-terminal (right) ends on the x-axis. Parametric lod scores are on the y-axis and are correlated to the genetic distance of the human chromosome on the x-axis. Lod scores >2 are seen at chromosome 20p11.2-12.

Table 1. Clinical information of 13 members of family 1

Family	Member	Gender	Age at onset	Age	Height (cm)	Weight (kg)	Plasma glucose (mmol/L) fasting	HbA1c (%)	Insulin (μ U/mL) fasting	Chol (mmol/L)	Trig (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	apoA (mmol/L)	apoB (mmol/L)
1	16	F	53	58	150	58.4	11.2	9.9	13.2	5.75	2.35	1.38	3.38	1.77	0.94
1	12	M	-	56	166	58.4	5.3	5.6	16.8	5.43	1.07	1.02	3.41	1.32	0.88
1	9	M	-	53	155	60	6	5.7	16.3	4.2	4.63	0.86	2.22	1.12	0.79
1	4	F	41	51	159	60.9	13.7	9.4	10	6.52	2.02	1.47	3.67	1.88	1.03
1	3	F	38	48	155	55	11.3	8.5	14.3	4.39	1.94	0.89	2.41	1.23	0.8
1	2	M	48	55	159	52.7	6.2	8	15.3	5.58	0.85	1.31	3.32	1.57	0.88
1	5	F	42	45	151	70	8.6	6.2	8.9	4.94	1.74	0.98	2.96	1.27	0.89
1	11	F	43	44	158	55	7	6.5	16.9	4.78	1.48	0.9	2.85	1.06	0.81
1	6	M	-	42	-	-	6.2	6.5	17.8	5.17	1.85	1.21	2.96	1.4	0.81
1	19	M	-	31	168	80	5.77	4.5	18	4.97	1.15	0.98	3.21	1.12	0.86
1	7	M	-	51	165	72	6.2	5.5	16.1	5.01	1.66	0.93	3.01	1.19	0.83
1	1	F	19		158	37	6.5*	5.4	16.1	2.54	0.76	0.83	1.23	0.95	0.47
1	15	F	-	7	106	19	4.99*	5	16	4.38	0.58	1.3	2.36	1.38	0.7

*Taking metformin for one year

affected and 6 unaffected members was extracted from whole blood using the standard phenol/chloroform method.

Plasma samples from family members were examined in the laboratory. Plasma glucose was examined by the hexokinase method (interassay coefficient of variation = 1.5%). HbA1c was determined by high-performance liquid chromatography on a Diamat Analyser (Bio-Rad, München, Germany). Plasma lipids, fibrinolysis and coagulation parameters, and albuminuria were measured by routine methods (22, 23). Insulin was examined with a commercial radioimmunoassay (Cambridge Biomedical).

Linkage analysis, using the multipoint genome-wide linkage approach (parametric and non-parametric), was performed on data from the 16 members of the family (Fig. 1) using data generated from the Human Omni ZhongHua-8 Bead Chip (Illumina) containing 650,632 SNP markers. SNPs with a call rate > 98% were used in the analysis. Genotypes of the 6802 Tag SNPs, distributed every 0.5cM across the genome without linkage disequilibrium, were chosen for the calculation of lod scores using the parametric or nonparametric linkage analysis package Merlin v. 1.1.23. The inheritance model was assumed to be dominant with 95% penetrance. The disease allele frequency was assumed to be 0.0005. Haplotypes were created with Merlin and viewed on HaploPainter. Lod score graphs were generated with Easy Linkage software. Lod scores >2 were considered to be above the suggestive threshold for linkage (24).

Figure 3. Pedigree of a family with apparent autosomal dominant inheritance of T2DM with female-related penetrance on chromosome 20. A haplotype consisting of 19 single nucleotide polymorphism markers on chromosome 20p11.2-12 (23.5~30.8cM) segregates with all affected individuals with T2DM. Affected individuals are shaded black.

Candidate genes were acquired by submitting the chromosome region (observed linkage peak) to the UCSC database. Coding sequences of candidate genes were amplified from genomic DNA using gene-specific primers. PCR products were sequenced in both directions with an ABI 3730 sequencer.

RESULTS

We identified a 3-generation Chinese family where T2DM segregates with female-related penetrance (Fig. 1). The clinical phenotypes and clinical assessments scores for individuals within this family were evaluated (Table 1). The proband had an early onset of T2DM at an age of 19 years (Number 1, Fig. 1). Affected individuals had histories of hyperglycemia (Plasma glucose >7 mmol/L, fasting) with normal fasting insulin levels. The average age

at onset of diabetes was 37 ± 5 years old. Morbidity of T2DM within this family was 50% (8/16).

Parametric multipoint Linkage analysis for T2DM revealed two maximum multipoint lod scores, of 2.1 on chromosome 20p11.2-12 between 23.5~30.8cM (Fig. 2) and of 2.36 on chromosome 16q22-23 between 91.24~91.61cM (Fig. S1). The interval of the former locus overlaps with that reported for the Alagille syndrome locus (25). However, non-parametric multipoint linkage analysis did not identify any region with lod scores >2. For the X chromosome, we also failed to identify region with lod scores >2. Haplotype analysis demonstrated 28 contiguous single nucleotide polymorphisms (SNPs) on chromosome 16q22-23 between rs13329908 and rs6564809 (Fig. S2), while 19 contiguous SNPs on chromosome 20 between rs1005351 and rs17189871 that were in common to all individuals with T2DM in this family (Fig. 3).

DISCUSSION

The linkage region on chromosome 20p11.2-12 contains 8 known or predicted genes. The coding sequences of these 8 genes (PLCB1, PLCB4, LAMP5, PAK7, ANKEF1, SNAP25, SLX4IP, and JAG1) were sequenced from 2 affected individuals (Numbers 1 and 3, Fig. 1). No coding sequence mutations were identified in these two individuals. No genes have been identified in the linkage region on chromosome 16.

Figure S1. Linkage of T2DM to chromosome 16q22-23. Genome-wide parametric multipoint lod score profile for family 1 with T2DM based on data generated with the Human Omni ZhongHua-8 Bead Chip array. Chromosome 16 is displayed from its p-terminal (left) to q-terminal (right) ends on the x-axis. Parametric lod scores are on the y-axis and are correlated to the genetic distance of the human chromosome on the x-axis. Lod scores >2 are seen on chromosome 16q22-23.

Since all of members of this Chinese family were exposed to the same environment, the identification of a high prevalence of T2DM in this family supports a strong genetic basis for this disease. Although T2DM in this family has the characteristics of being female-related, no evidence was found for genes on the sex chromosome in the etiology of this family. When a multipoint genome-wide linkage analysis was performed on SNP data from the 16 members of this family, a genomic region on chromosome 20p11.2-12 was identified, a genomic region which had previously not been linked to T2DM. The genetic bases of some diabetic complication have recently been described, including diabetic kidney disease, retinopathy, neuropathy and accelerated cardiovascular disease (22). Symptoms of these diabetic complications were not found in the affected members of this family by self-reporting, and none of the genes identified for these disorders are located within our candidate region for T2DM on chromosome 20p11.2-12.

One of the most interesting genes in this region, SNAP25 (synaptosome-associated protein of 25 kD), has been reported to play a key role in insulin secretion

Figure S2. Pedigree of a family with apparent autosomal dominant inheritance of T2DM with female-related penetrance on chromosome 16. A haplotype consisting of 11 single nucleotide polymorphism markers on chromosome 16q22-23 (91.24–91.61cM, lilac) segregates with all affected individuals with T2DM. Affected individuals are shaded black. The proband is indicated with an oblique arrow.

(26). SNAP25 is a presynaptic plasma membrane protein that functions in synaptic vesicle membrane docking and in the fusion pathway. Truncated SNAP-25 (1–197), like botulinum neurotoxin A, inhibits insulin secretion from HIT-T15 insulinoma cells (27). Expression of SNAP25 correlates negatively with *in vivo* measurements of HbA1c levels and positively to glucose stimulated insulin secretion (GSIS) *in vitro* in human islets, with decreased levels of expression of this gene being associated with T2DM pathogenesis (28). A heterozygous SNP at SNAP25 (rs362551) shows a lower risk of metabolic syndrome (MetS) compared with the reference group, which implies that this gene/SNP plays a role in MetS development (29).

Although we did not detect any coding mutations in the sequences of the SNAP-25 gene in our samples, it is possible that mutations, including insertions or deletions, in noncoding regulatory regions might alter SNAP-25 expression and be responsible for the T2DM phenotype. A study by Andersson *et al.* also failed to detect any SNPs associated with reduced expression of SNAP25 in human islets that were positively correlated with GSIS (28). An alternative possibility is that one of the other remaining 7 candidate genes in the linkage region at chromosome 20p11.2-12 might cause T2DM in this family.

An increased prevalence of T2DM was noted in females of this Chinese family; however, it may not be associated with X chromosome variants as all sites on this chromosome had low scores (Table S3). Previous studies have also described sex-related variation in T2DM. For example, in a study of Canadian immigrants, with 1,122,771 participants, a greater incidence of diabetes was detected in females (30). T2DM has been widely studied through linkage and association analysis, which have identified a large number of susceptibility loci, however, none of these previously identified loci included the genomic region chromosome 20p11.2-12 identified in our study. This newly identified region overlaps with the locus reported for the Alagille syndrome, a syndrome associated with a defect in the notch signalling pathway (31). Defects in notch signalling are also correlated with insulin resistance in hepatocyte (32).

Family-based studies, like ours, have a high likelihood of detecting rare or very rare variants. Additional studies are needed to determine whether genetic changes in non-coding regions are responsible for the high female-to-male ratio of T2DM in this Chinese family.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

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