Diabetologia (2007) 50:2461–2466
DOI 10.1007/s00125-007-0827-5

ARTICLE

Variations in the *HHEX* gene are associated with increased risk of type 2 diabetes in the Japanese population

M. Horikoshi · K. Hara · C. Ito · N. Shojima · R. Nagai · K. Ueki · P. Froguel · T. Kadowaki

Received: 8 June 2007 / Accepted: 7 August 2007 / Published online: 10 October 2007

Abstract
Aims/hypothesis Recently, several groups have carried out whole-genome association studies in European and European-origin populations and found novel type 2 diabetes-susceptibility genes, fat mass and obesity associated (*FTO*), solute carrier family 30 (zinc transporter), member 8 (*SLC30A8*), haematopoietically expressed homeobox (*HHEX*), exostoses (multiple) 2 (*EXT2*), CDK5 regulatory subunit associated protein 1-like 1 (*CDKAL1*), cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4) (*CDKN2B*) and insulin-like growth factor 2 mRNA binding protein 2 (*IGF2BP2*), which had not been in the list of functional candidates. The aim of this study was to determine the association between single nucleotide polymorphisms (SNPs) in these genes and type 2 diabetes in participants from the Japanese population.
Methods Sixteen previously reported SNPs were genotyped in 864 Japanese type 2 diabetes individuals (535 men and 329 women; age 63.1±9.5 years (mean±SD), BMI 24.3±3.9 kg/m^2) and 864 Japanese control individuals (386 men and 478 women; age 69.5±6.8 years, BMI 23.8±3.7 kg/m^2).
Results The SNPs rs5015480 [odds ratio (OR)=1.46 (95% CI 1.20–1.77), $p=2.0\times10^{-4}$], rs7923837 [OR=1.40 (95% CI 1.17–1.68), $p=2.0\times10^{-4}$] and rs1111875 [OR=1.30 (95% CI 1.11–1.52), $p=0.0013$] in *HHEX* were significantly associated with type 2 diabetes with the same direction as previously reported. SNP rs8050136 in *FTO* was nominally associated with type 2 diabetes [OR=1.22 (95% CI 1.03–1.46), $p=0.025$]. SNPs in other genes such as rs7756992 in *CDKAL1*, rs10811661 in *CDKN2B* and rs13266634 in *SLC30A8* showed nominal association with type 2 diabetes. rs7756992 in *CDKAL1* and rs10811661 in *CDKN2B* were correlated with impaired pancreatic beta cell function as estimated by the homeostasis model assessment beta index ($p=0.023$, $p=0.0083$, respectively).
Conclusions/interpretation *HHEX* is a common type 2 diabetes-susceptibility gene across different ethnic groups.

Keywords Japanese population · Single nucleotide polymorphism · Susceptibility gene · Type 2 diabetes

M. Horikoshi and K. Hara contributed equally to this study.

Electronic supplementary material The online version of this article (doi:10.1007/s00125-007-0827-5) contains supplementary material, which is available to authorised users.

M. Horikoshi · K. Hara · N. Shojima · K. Ueki · T. Kadowaki (✉)
Department of Metabolic Diseases,
Graduate School of Medicine,
University of Tokyo,
Hongo 7-3-1,
Bunkyo-ku, Tokyo 113-8655, Japan
e-mail: kadowaki-3im@h.u-tokyo.ac.jp

K. Hara · R. Nagai · T. Kadowaki
Department of Clinical Genome Informatics,
Tokyo University Hospital,
Tokyo, Japan

C. Ito
Medical Court Life Care Clinic,
Hiroshima, Japan

R. Nagai
Department of Cardiovascular Medicine,
Graduate School of Medicine, University of Tokyo,
Tokyo, Japan

P. Froguel
CNRS UMR 8090, Institut Pasteur de Lille,
Lille Cedex, France

Abbreviations

HOMA-beta	homeostasis model assessment of beta cell function
HOMA-IR	homeostasis model assessment of insulin resistance
LD	linkage disequilibrium
MAF	minor allele frequency
OR	odds ratio
SNP	single nucleotide polymorphism

Introduction

Type 2 diabetes is a complex disease; multiple genes are involved in its onset and development. To date, a number of genes have been reported to be associated with type 2 diabetes. Most of the genes are investigated because they are presumed to be relevant to the pathogenesis of type 2 diabetes based on the function of the gene. However, because the whole picture of the pathogenesis of type 2 diabetes is still to be clarified, this 'candidate-gene approach' is limited in power to detect novel disease-susceptibility genes.

Due to the recent development of single nucleotide polymorphism (SNP) typing technology and accumulation of the information on the linkage disequilibrium (LD) of the human genome, whole-genome association studies have now become a feasible way of searching for a novel disease-susceptibility gene across the whole genome without prior information as to the function of the gene. Indeed, since the original discovery of the powerful type 2 diabetes gene, transcription factor 7-like 2 (T cell specific, HMG-box) (*TCF7L2*), by a joint group from Iceland, the USA and Denmark [1], many groups have confirmed the significant association between type 2 diabetes and this gene in several populations [2–4]. Following the detection of *TCF7L2*, a number of groups, including French-Canadian [5], Icelandic [6], UK [7, 8], Finnish [9] and Finnish–Swedish [10], have described new type 2 diabetes-susceptibility genes obtained by whole-genome association study. These genes are solute carrier family 30 (zinc transporter), member 8 (*SLC30A8*), haematopoietically expressed homeobox (*HHEX*), exostoses (multiple) 2 (*EXT2*), CDK5 regulatory subunit associated protein 1-like 1 (*CDKAL1*), fat mass and obesity associated (*FTO*), cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4) (*CDKN2A*), cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4) (*CDNK2B*) and insulin-like growth factor 2 mRNA binding protein 2 (*IGF2BP2*), which have not been well-documented and thus not thought to be good candidates for type 2 diabetes genes.

It is well known that frequencies of genetic variations are different among ethnic groups, leading to differences in the effect and importance of the same susceptibility gene according to the different ethnic groups. It is especially important to investigate this in populations in Eastern Asia, including Japan and China, where the number of individuals with type 2 diabetes is increasing rapidly. Therefore, we have investigated whether the confirmed type 2 diabetes variants in these genes in Europid populations are also associated with type 2 diabetes in participants from the Japanese population.

Methods

Participants We performed an association study in 864 Japanese type 2 diabetes individuals and 864 Japanese non-diabetic individuals. The clinical characteristics are described in Electronic supplementary material (ESM) Table 1. The diabetic patients were randomly recruited from among those attending the outpatient clinic of the Department of Metabolic Diseases, Graduate School of Medicine, University of Tokyo (Tokyo, Japan), and the non-diabetic individuals from among those undergoing annual health check-ups at the Hiroshima Atomic Bomb Casualty Council Health Management Center (Hiroshima, Japan) [3]. Details of the inclusion and exclusion criteria of our case–control samples are described in the ESM. In order to evaluate the possibilities of population stratification in our case–control samples, we chose 50 SNPs from the database of International HapMap project that are not in LD with each other and genotyped them in 382 Tokyo cases and 382 Hiroshima controls. Then, we investigated whether there was inflation of the number of SNPs with $p<0.05$ for association between the 50 SNPs and type 2 diabetes. As described in ESM Table 2, there was no evidence of inflation of the number of SNPs with $p<0.05$. The number of SNPs with $p<0.05$ was 2, whereas the expected number of SNPs with $p<0.05$ was 2.35 (47×0.05, three SNPs were not variants in the Japanese population). All the participants gave informed consent, and the Ethics Committee of the University of Tokyo approved this study.

Selection of SNPs genotyped in this study We selected all the SNPs in genes that were repeatedly confirmed as type 2 diabetes-susceptibility variants in the recent six association studies [5–10]. SNPs that reside in a gene which were reported only in a single study were not included in this study, e.g. SNPs in *LOC387761*, chromosome 4 open reading frame 32 (*FLJ39370*, also known as *C4orf32*) and protein kinase N2 (*PKN2*). SNPs that we had previously reported were also excluded, e.g. SNP rs7903146 in *TCF7L2* and rs1801282 in peroxisome proliferator-activated receptor gamma (*PPARG*). As for *CDKAL1*, SNP rs7754840 was not included as it was only 216 bp downstream of rs10946398, and these were in LD with each other. SNP

rs5219 of potassium inwardly-rectifying channel, subfamily J, member 11 (*KCNJ11*) was also excluded because it was within 1 kb of rs5215. All together, 16 SNPs were investigated in this study.

Genotyping SNPs Among the 16 SNPs, *FTO* rs9939609 was genotyped by direct sequencing, performed with a BigDye Terminator (Applied Biosystems, Foster City, CA, USA) and resolved using an ABI 3700 automated DNA sequencer (Applied Biosystems). The rest of the SNPs were genotyped using Taqman SNP genotyping assays by means of an ABI 7900HT (Applied Biosystems) according to the manufacturer's protocol. Genotyping success rates ranged between 97.7% (rs4402960) and 100% (rs9939609). Concordance rate, based on duplicate comparisons in 192 control participants and 192 type 2 diabetes patients, was 100%. Samples with ambiguous base calling were genotyped twice. All the SNPs were in accord with Hardy–Weinberg equilibrium in type 2 diabetes participants ($p>0.09$) and control participants ($p>0.17$).

Evaluating LD The r^2 values between SNPs were estimated using a software package based on the expectation-maximisation algorithm, SNPAlyze version 3.5.0 (Dynacom, Tokyo, Japan). The LD structure was plotted using GOLD software (http://www.sph.umich.edu/csg/abecasis/GOLD/).

Biological measurements Insulin resistance and pancreatic beta cell function were quantified using homeostasis model assessment (HOMA-IR and HOMA-beta, respectively); HOMA-IR=fasting insulin (pmol/l)×glucose (mmol/l)/22.5×6 (correction for μU/l in original formula) and HOMA-beta=fasting insulin (pmol/l)×20/glucose (mmol/l) −3.5×6 (correction for μU/l in original formula) as widely used [11]. Data were expressed as means±SD. Details for the methods of other biological measurements are described in the ESM.

Statistical analysis The proportions of genotypes or alleles were compared between type 2 diabetic and non-diabetic participants using a multiple logistic regression analysis adjusted for age and sex. Differences in HOMA index according to genotypes were determined by analysis of covariates in non-diabetic participants, after adjustment for age and sex. The statistical analyses were performed using JMP for Windows version 6.00 software (SAS Institute, Cary, NC, USA). Values of p were corrected by Bonferroni adjustment and $p<0.0031$ (0.05 divided by 16, the total number of SNPs studied), was considered significant. The allele-specific odds ratio (OR) was assessed by counting the number of risk alleles for each individual, and we used the number of risk alleles to predict case/control status using logistic regression.

Results

The association results of the type 2 diabetes-susceptibility variants in the present study are shown in Table 1. Among the 16 SNPs from eight genes, all three SNPs, rs5015480 [OR=1.46 (95% CI 1.20–1.77), $p=2.0\times10^{-4}$], rs7923837 [OR=1.40 (95% CI 1.17–1.68), $p=2.0\times10^{-4}$] and rs1111875 [OR=1.30 (95% CI 1.11–1.52), $p=0.0013$] in *HHEX* were significantly associated with type 2 diabetes in the Japanese population after adjustment for multiple testing. The OR for SNP rs5015480 was the highest in the present study. The risk alleles were all minor alleles and the associations were in the same direction as the previous studies [5, 8–10]. We determined the LD pattern in this region and found that this region was separated into two LD blocks in our samples; one containing insulin degrading enzyme (*IDE*) and kinesin family member 11 (*KIF11*), and the other containing *HHEX* (ESM Fig. 1). All three SNPs showed nominal association with BMI (rs5015480 TT vs TC vs CC, 24.0±0.15 vs 23.3±0.24 vs 22.1±0.92, $p=0.011$; rs7923837 AA vs AG vs GG, 24.0±0.16 vs 23.3±0.23 vs 23.9±0.70, $p=0.037$; rs1111875 TT vs TC vs CC, 24.1±0.17 vs 23.4±0.20 vs 23.3±0.49, $p=0.042$). When we stratified the participants into obese (BMI≥25 kg/m^2) and non-obese (BMI <25 kg/m^2) participants, each SNP showed a higher OR in the obese participants [OR=1.91 (95% CI 1.34–2.75), $p=5.0\times10^{-4}$; OR=1.58 (95% CI 1.15–2.17), $p=0.0049$; OR=1.51 (95% CI 1.16–1.98), $p=0.0025$, respectively] (ESM Table 3) than in non-obese participants. The associations were negative or only nominally significant in participants with BMI <25 kg/m^2 ($p=0.029$, $p=0.011$ and $p=0.080$, respectively).

Among the two SNPs in the *FTO* gene, only rs8050136 showed a nominal significance in terms of the association with type 2 diabetes [OR=1.22 (95% CI 1.03–1.46), $p=0.025$]. rs8050136 was not associated with BMI (CC 23.8±0.16 vs CA 23.7±0.23 vs AA 23.8±0.49, $p=0.99$), and the nominal association between rs8050136 and type 2 diabetes was not strengthened after correction for BMI [OR=1.22 (95% CI 1.03–1.46), $p=0.025$ adjusted for age, sex and BMI]. The other SNP in the *FTO* gene, rs9939609, was identified as a type 2 diabetes-susceptibility variant that predisposes to diabetes through an effect on BMI in the UK population [7]. However, we could not detect any significant association between rs9939609 and type 2 diabetes or with BMI (TT 24.1±0.17 vs TA 24.6±0.22 vs AA 24.3±0.62, $p=0.17$). No other clinical parameters related to type 2 diabetes such as HbA_{1c}, fasting glucose, fasting insulin, HOMA-IR, and HOMA-beta were associated with rs9939609.

SNPs in *CDKAL1*, *CDKN2B* and *SLC30A8* genes showed nominally significant association with type 2 diabetes. As for *EXT2*, *IGF2BP2* and *KCNJ11*, we could not detect any SNPs that were significantly associated with type 2 diabetes,

Table 1 Association results for confirmed type 2 diabetes-susceptibility variants in the Japanese population

SNP	Chr	Region	Major allele (A)	Minor allele (a)	Type 2 diabetes		Control		Genotype-specific *p* value	OR (95% CI)	Allele-specific *p* value	Reference
					MAF	AA/Aa/aa	MAF	AA/Aa/aa				
rs1111875	10	*HHEX*	T	C[a]	0.326	394/371/95	0.265	461/342/57	0.0053	1.30 (1.11–1.52)	0.0013	[5, 8–10]
rs7923837	10	*HHEX*	A	G[a]	0.237	496/317/45	0.187	564/264/28	0.0010	1.40 (1.17–1.68)	2.0×10^{-4}	[5]
rs5015480	10	*HHEX*	T	C[a]	0.197	549/279/29	0.146	626/219/16	8.0×10^{-4}	1.46 (1.20–1.77)	2.0×10^{-4}	[8]
rs8050136	16	*FTO*	C	A[a]	0.238	486/334/37	0.200	554/269/38	0.022	1.22 (1.03–1.46)	0.025	[8, 9]
rs9939609	16	*FTO*	T	A[a]	0.220	528/298/38	0.190	573/251/40	0.27	1.10 (0.92–1.32)	0.27	[7]
rs7756992	6	*CDKAL1*	A	G[a]	0.514	238/426/188	0.471	191/450/216	0.027	1.20 (1.03–1.39)	0.017	[6]
rs10946398	6	*CDKAL1*	A	C[a]	0.465	239/434/179	0.429	280/423/158	0.16	1.16 (1.00–1.34)	0.55	[8]
rs10811661	9	*CDKN2B*	T[a]	C	0.387	324/408/129	0.434	290/398/176	0.018	1.22 (1.05–1.41)	0.0076	[8–10]
rs564398	9	*CDKN2B*	T	C[a]	0.164	609/221/31	0.150	623/207/25	0.70	1.08 (0.89–1.32)	0.41	[8]
rs1113132	11	*EXT2*	C[a]	G	0.355	360/390/110	0.381	326/411/122	0.21	1.12 (0.96–1.30)	0.14	[5]
rs11037909	11	*EXT2*	T[a]	C	0.349	366/393/105	0.376	330/417/116	0.21	1.13 (0.97–1.31)	0.12	[5]
rs3740878	11	*EXT2*	A[a]	G	0.357	352/403/106	0.388	318/418/125	0.23	1.14 (0.98–1.33)	0.089	[5]
rs1470579	3	*IGF2BP2*	A	C[a]	0.372	324/432/104	0.345	372/378/107	0.039	1.12 (0.96–1.30)	0.16	[10]
rs4402960	3	*IGF2BP2*	G	T[a]	0.323	384/380/84	0.309	407/348/86	0.65	1.05 (0.90–1.23)	0.55	[8–10]
rs13266634	8	*SLC30A8*	C[a]	T	0.396	328/383/149	0.430	293/394/172	0.055	1.19 (1.03–1.37)	0.016	[5, 6, 8–10]
rs5215	11	KCNJ11	T	C[a]	0.382	334/393/131	0.373	332/417/113	0.56	1.03 (0.89–1.20)	0.68	[8]

The SNPs are shown with the risk allele and MAF and the exact count of each genotype in type 2 diabetes and controls. Genotype-specific *p* values are adjusted for age and sex. Risk allele-specific ORs and *p* values are calculated using an additive genetic model that in logistic regression is multiplicative on the OR scale. The OR for each SNP was adjusted simultaneously for age and sex.
Chr chromosome
[a] Risk allele

but the associations were in the same direction as the previous reports in the European populations.

When we tested the SNPs for quantitative trait association in non-diabetic participants, we found several SNPs to be nominally associated with pancreatic beta cell function as estimated by HOMA-beta. Participants with the risk allele of rs7756992 in *CDKAL1* ($p=0.023$), rs10811661 in *CDKN2B* ($p=0.0083$), rs11037909 ($p=0.0353$) and rs3740878 ($p=0.022$) in *EXT2*, and rs1470579 ($p=0.0446$) and rs4402960 ($p=0.0096$) in *IGF2BP2* had lower HOMA-beta values (Fig. 1). However, when taking the multiple comparisons into account, none of the SNPs reached significance. As for HOMA-IR, none of the SNPs was associated with HOMA-IR.

Discussion

Recent reports have revealed novel type 2 diabetes-susceptibility genes, such as *SLC30A8*, *CDKAL1*, *CDKN2A/2B*, *IGF2BP2*, *EXT2*, *HHEX* and *FTO*, in the European population [5–10]. In this study, we confirmed the significant association of *HHEX* with type 2 diabetes in the Japanese population. The OR values of the three SNPs genotyped in *HHEX* (1.20–1.46) were higher than those of the European population (1.20). However, there was a noticeable difference in the frequencies of the risk alleles; 0.24–0.33 in the Japanese populations and 0.65 in Europids, which was also observed for the *TCF7L2* gene variants in our previous report [3]. We observed a nominal inverse

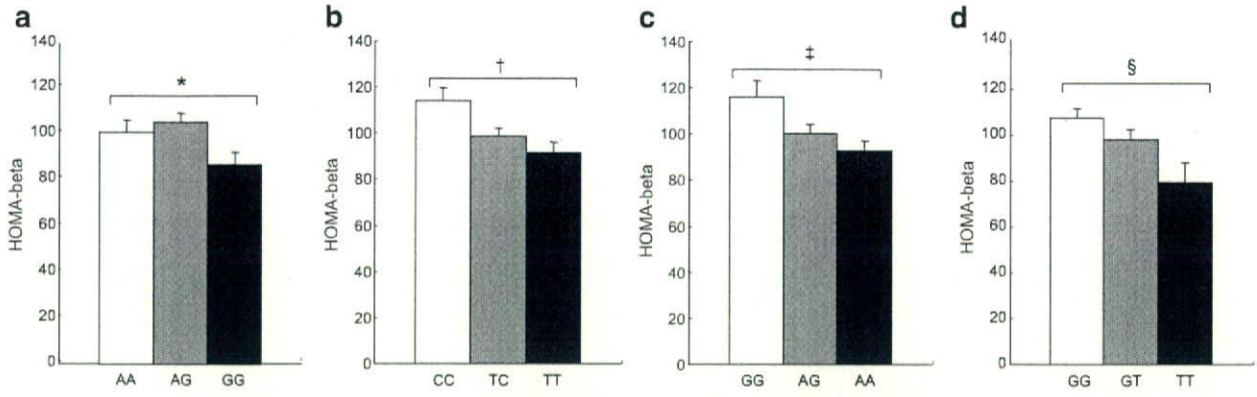

Fig. 1 Association between variants in *CDKAL1* rs7756992 (*p=0.023) (**a**), *CDKN2B* rs10811661 ($^{\dagger}p$=0.0083) (**b**), *EXT2* rs3740878 ($^{\ddagger}p$=0.022) (**c**), and *IGF2BP2* rs4402960 ($^{\S}p$=0.0096) (**d**) with pancreatic beta cell function as estimated by HOMA-beta index. Associations between SNPs representing the four genes and the indices of pancreatic beta cell function are shown. Associations were assessed in non-diabetic participants

association between the SNPs in *HHEX* and BMI in the control participants (p=0.011–0.042). Adjustment for BMI did not strengthen the association between SNPs in *HHEX* and type 2 diabetes. Haplotype analysis of the *HHEX* gene did not strengthen the association, either (p=0.0013). However, when we performed a stratified association study, the ORs grew higher in the group of obese participants. This may indicate that the risk alleles of *HHEX* do not predispose to diabetes through an effect on BMI, but it may predispose to diabetes under obesity-inducing circumstances. Sladek et al. [5] reported that multiple SNPs within an extended region containing *IDE*, *KIF11* and *HHEX* were associated with type 2 diabetes due to preserved LD in this region. In our samples, LD in this region was not relatively preserved and *HHEX* represents one block of strong LD (ESM Fig. 1), suggesting that an SNP in *HHEX* is the functional SNP conferring type 2 diabetes susceptibility. *HHEX* has been reported to be essential for beta cell function and development and is a target of the wingless-type MMTV integration site family (*WNT*) signalling pathway, as is *TCF7L2* [5].

FTO, *CDKAL1*, *CDKN2B* and *SLC30A8* showed nominal association with type 2 diabetes. *FTO* was identified as a type 2 diabetes-susceptibility variant that predisposes to diabetes through an effect on BMI in the UK population [7]. As to possible reasons for not detecting association between BMI and *FTO* SNPs in the Japanese population, this may be due to the fact that our samples were from a less obese Japanese population compared with Europids. Indeed, mean BMI of participants with the risk genotype in UK non-diabetic controls is 25.4–27.1 kg/m^2, whereas that of Japanese non-diabetic controls is 24.3 kg/m^2. The Japanese population has similar prevalence of type 2 diabetes to Europids, whereas the prevalence of obesity in the Japanese population is much lower, indicating that the Japanese population has a predisposition to type 2 diabetes under a lower burden of obesity compared with the Europid. Therefore, it is possible that the present study could not detect the effect of the *FTO* gene on BMI in the Japanese population, a less obese population than the Europid.

SNP rs7756992 in *CDKAL1* is the SNP that is reportedly associated with type 2 diabetes in Han Chinese individuals from Hong Kong [6]. The minor allele frequency (MAF) of rs7756992 in the Han Chinese was higher than that in the European-origin population and the MAF in the Japanese was very similar to that of the Han Chinese. We detected a nominally significant association between this SNP and Japanese type 2 diabetes [OR=1.20 (95% CI 1.03–1.39), p=0.017]. Given that *CDKAL1* and *CDKN2B* showed nominal association with impaired pancreatic beta cell function as estimated by HOMA-beta index in the present study, and that they may well have some role in transduction of glucose toxicity or regenerative capacity of pancreatic beta cells [6], they could be good candidate genes for Japanese type 2 diabetes. *EXT2* and *IGF2BP2* also showed nominal association with impaired pancreatic beta cell function, but we could not detect any significant association with type 2 diabetes.

The absence of significant association is often attributed to lack of power. The present study had 80% power to detect an OR of 1.20, when the frequency of a risk allele was 30%. However, the power to detect an OR of 1.12, the smallest OR in the whole-genome association studies [8–10], was 50%. Also, the characterisation of the control sample lacks OGTT data. These facts should be regarded as limitations of the present study.

It has been reported that life-style alteration can reduce the risk of type 2 diabetes, even in individuals carrying the type 2 diabetes-susceptibility variant of *TCF7L2* [2]. Frequencies of risk alleles of three SNPs in *HHEX* were lower in our samples than in European populations but

effects of those SNPs on susceptibility to diabetes were stronger. Therefore, genotyping SNPs of *HHEX* in an individual may be effective for a personalised preventive medicine in Asian populations.

In conclusion, this is the first report to describe a significant association between type 2 diabetes and *HHEX* in the Asian population. Further replication of these studies in Eastern Asian populations who share a quite similar genetic background are awaited.

Acknowledgements This work was supported by a Grant-in-aid from the Ministry of Health, Labour and Welfare (to K. Hara and T. Kadowaki) and by a Grant-in-aid for the 21st Century COE Programme (to R. Nagai). We thank N. Miyama for her technical assistance.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

References

1. Grant SF, Thorleifsson G, Reynisdottir I et al (2006) Variant of transcription factor 7-like 2 (*TCF7L2*) gene confers risk of type 2 diabetes. Nat Genet 38:320–323
2. Florez JC, Jablonski KA, Bayley N et al (2007) *TCF7L2* polymorphisms and progression to diabetes in the Diabetes Prevention Program. N Engl J Med 355:241–250
3. Horikoshi M, Hara K, Ito C et al (2007) A genetic variation of the transcription factor 7-like 2 gene is associated with risk of type 2 diabetes in the Japanese population. Diabetologia 50:747–751
4. Cauchi S, El Achhab Y, Choquet H et al (2007) *TCF7L2* is reproducibly associated with type 2 diabetes in various ethnic groups: a global meta-analysis. J Mol Med 85:777–782
5. Sladek R, Rocheleau G, Rung J et al (2007) A genome-wide association study identifies novel risk loci for type 2 diabetes. Nature 445:881–885
6. Steinthorsdottir V, Thorleifsson G, Reynisdottir I et al (2007) A variant in *CDKAL1* influences insulin response and risk of type 2 diabetes. Nat Genet 39:770–775
7. Frayling TM, Timpson NJ, Weedon MN et al (2007) A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity. Science 316:889–894
8. Zeggini E, Weedon MN, Lindgren CM et al (2007) Replication of genome-wide association signals in UK. Samples reveals risk loci for type 2 diabetes. Science 316:1336–1341
9. Scott LJ, Mohlke KL, Bonnycastle LL et al (2007) A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 316:1341–1345
10. Saxena R, Voight BF, Lyssenko V et al (2007) Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. Science 316:1331–1336
11. Matthew DR, Hosker JP, Rudenski AS et al (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419

Frequency of the G/G Genotype of Resistin Single Nucleotide Polymorphism at −420 Appears to Be Increased in Younger-Onset Type 2 Diabetes

Masaaki Ochi,[1] Haruhiko Osawa,[1] Yushi Hirota,[2] Kazuo Hara,[3] Yasuharu Tabara,[4] Yoshiharu Tokuyama,[5] Ikki Shimizu,[6] Azuma Kanatsuka,[5] Yasuhisa Fujii,[6] Jun Ohashi,[7] Tetsuro Miki,[8] Naoto Nakamura,[9] Takashi Kadowaki,[3] Mitsuo Itakura,[10] Masato Kasuga,[2] and Hideichi Makino[1]

OBJECTIVE—Resistin is an adipocyte-secreted cytokine associated with insulin resistance in mice. We previously reported that the G/G genotype of a resistin single nucleotide polymorphism (SNP) at −420 increases type 2 diabetes susceptibility by enhancing its promoter activity. The aim of the present study was to determine the relevance of SNP −120 in a large number of subjects.

RESEARCH DESIGN AND METHODS— We examined 2,610 type 2 diabetic case and 2,502 control subjects. The relation between SNP −420 and the age of type 2 diabetes onset was further analyzed by adding 237 type 2 diabetic subjects with age of onset ≤40 years.

RESULTS—When analyzed without considering subject age, the SNP −420 genotype was not associated with type 2 diabetes. Since we reported that the onset of type 2 diabetes was earlier in G/G genotype, we analyzed the data using a trend test for age intervals of 10 years. The frequency of G/G genotype differed among age grades in type 2 diabetes ($P = 0.037$) and appeared to be higher in younger grades. In type 2 diabetes, G/G genotype was more frequent in subjects aged <40 years than in those aged ≥40 years (G/G vs. C/C, $P = 0.003$). In a total of 2,430 type 2 diabetic subjects with age of onset <60 years, the trend test showed that the G/G genotype had an increasing linear trend as the age grade of type 2 diabetes onset became younger ($P = 0.0379$). In control subjects, the frequency of C/G genotype showed an increasing linear trend with increasing age ($P = 0.010$).

CONCLUSIONS—The G/G genotype frequency of resistin SNP −420 appears to be increased in younger-onset type 2 diabetic subjects. ***Diabetes* 56:2834–2838, 2007**

One characteristic of type 2 diabetes is insulin resistance in insulin target tissues (1). Type 2 diabetes is a probable polygenic disease, and its major genetic factors have yet to be identified (2). Single nucleotide polymorphisms (SNPs) such as peroxisome proliferator–activated receptor (PPAR)γ, KCNJ11, and TCF7L2 have been reported to be associated with type 2 diabetes (3). We reported that SNP at −420 in the resistin gene (*RETN*) (rs1862513) is associated with type 2 diabetes (4).

In mice, resistin is secreted from adipocytes and antagonizes insulin action both in vitro and in vivo (5,6). Serum resistin is increased in obese diabetic mice and is reduced by PPARγ ligands (6). Transgenic mice overexpressing *retn* in the liver have high serum resistin and are insulin resistant (7). The $retn^{-/-}$ mice show lower fasting blood glucose (8). Therefore, the role of resistin as an adipocyte-secreted cytokine inducing insulin resistance appears to be established in rodents.

In humans, *RETN* is rarely expressed in adipose tissues and is expressed at high levels in monocytes or macrophages, in contrast to its dominant expression in adipose tissues in mice (9,10). Macrophages infiltrating into adipose tissues could account for the observed insulin resistance in obese mice, suggesting a possible role of resistin in insulin resistance in humans (11,12). The role of *RETN* in human type 2 diabetes or obesity has been controversial in studies of the association of SNPs or serum resistin (4,13–16). The discrepancy among previous reports may be resolved by considering the SNP −420 genotype or by analyzing a larger number of samples.

We reported that the G/G genotype of *RETN* promoter SNP −420 is associated with type 2 diabetes susceptibility (4). Sp1 and Sp3 transcription factors specifically bind to the DNA element including −420G, resulting in an enhanced promoter activity. *RETN* mRNA in monocytes is positively associated with its simultaneous serum levels and is highest in subjects with G/G genotype (17). Serum resistin is higher in type 2 diabetic subjects than in control subjects and highest in subjects with G/G genotype, followed by C/G and C/C. Therefore, the specific recognition of −420G by Sp1/3 appears to increase *RETN* promoter

From the [1]Department of Molecular and Genetic Medicine, Ehime University Graduate School of Medicine, Ehime, Japan; the [2]Division of Diabetes, Digestive and Kidney Diseases, Department of Clinical Molecular Medicine, Kobe University Graduate School of Medicine, Kobe, Japan; the [3]Department of Metabolic Diseases, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; the [4]Department of Basic Medical Research and Education, Ehime University Graduate School of Medicine, Ehime, Japan; the [5]Diabetes Center, Chiba Central Medical Center, Chiba, Japan; [6]Department of Internal Medicine, Ehime Prefectural Hospital, Ehime, Japan; the [7]Department of Human Genetics, School of International Health, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; the [8]Department of Geriatric Medicine, Ehime University Graduate School of Medicine, Ehime, Japan; the [9]Department of Endocrinology and Metabolism, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan; and the [10]Division of Genetic Information, Institute for Genome Research, University of Tokushima, Tokushima, Japan.

Address correspondence and reprint requests to Haruhiko Osawa or Hideichi Makino, Department of Molecular and Genetic Medicine, Ehime University Graduate School of Medicine, Toon, Ehime 791-0295, Japan. E-mail: harosawa@m.ehime-u.ac.jp or hidemak@m.ehime-u.ac.jp.

Received for publication 17 August 2006 and accepted in revised form 8 August 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 13 August 2007. DOI: 10.2337/db06-1157.

Additional information for this article can be found in an online appendix at http://dx.doi.org/10.2337/db06-1157.

PPAR, peroxisome proliferator–activated receptor; SNP, single nucleotide polymorphism.

TABLE 1
G/G genotype was not associated with type 2 diabetes when age was not considered

Genotype or allele	Type 2 diabetic subjects	Control subjects	Comparison	χ^2	P	OR (95% CI)
n	2,610	2,502	—	—	—	—
CC	1,169	1,080	CC/CG/GG	1.44	0.486	—
CG	1,144	1,123	GG vs. CC	0.87	0.351	0.92 (0.77–1.10)
GG	297	299	CG vs. CC	1.04	0.308	0.94 (0.84–1.06)
			GG vs. CG	0.08	0.784	0.98 (0.81–1.17)
			GG + CG vs. CC	1.37	0.242	0.94 (0.84–1.05)
			GG vs. CG + CC	0.40	0.525	0.95 (0.80–1.12)
G-allele	1,738 (33.3)	1,721 (34.4)	G- vs. C-allele	1.38	0.241	—

Data are n or n (%) unless otherwise indicated. χ^2 test was used for statistical analysis. ORs calculated by defining the G-allele as a susceptibility allele.

activity, which could induce insulin resistance and human type 2 diabetes through enhanced monocyte mRNA and serum levels of resistin. Therefore, we analyzed the relevance of *RETN* SNP −420 in a large number of samples.

RESEARCH DESIGN AND METHODS

We recruited native Japanese subjects—2,610 type 2 diabetic case and 2,502 control subjects—from six prefectures located in Honshu and Shikoku in Japan. These samples are assumed not to be heterogeneous since Matsumoto et al. (18) showed that the Japanese population is homogenous, except for the Ainus from Hokkaido and the Okinawans from Miyako, using genetic markers of human immunoglobulin. Diabetes was diagnosed based on American Diabetes Association criteria (19). The control subjects were chosen based on either no history of diabetes and A1C levels <5.6% or normal glucose tolerance as evidenced by a 75-g oral glucose tolerance test. To analyze the relation between SNP −420 and age of type 2 diabetes onset, 237 type 2 diabetic patients with onset age ≤40 years were added. The clinical characteristics of the 2,610 type 2 diabetic case and 2,502 control subjects and additional 237 type 2 diabetic subjects are summarized in Supplementary Table 1 (available in an online appendix at http://dx.doi.org/10.2337/db06-1157). The average age of the control subjects was significantly older than the age of onset of type 2 diabetes in *panel 1* (Student's t test, $P < 0.0001$). Of subjects in *panel 1*, we typed SNP −420 in 397 type 2 diabetic patients and 406 control subjects as *panels 1* and *2* and 154 case and 143 control subjects as *panel 3* in a previous article (4).

All subjects were informed of the purpose of the study, and informed consent was obtained. The study was approved by the ethics committee of Ehime University (including Chiba Central Medical Center), Ehime Prefectural Hospital, Kobe University, the University of Tokyo, the University of Tokushima, and Kyoto Prefectural University of Medicine.

The statistical power was calculated as follows (20). We assume that S and N are susceptibility and normal alleles, respectively. When the genotype relative risk is assumed to be 1.20 for SN and 1.44 for SS, the population frequency of S is 30% as SNP −420 and the prevalence of diabetes is 6.9% based on the International Diabetes Federation Diabetes e-Atlas (http://www.eatlas.idf.org/About_e_Atlas/); the penetrance for genotypes of SS, SN, and NN were calculated to be 0.088, 0.074, and 0.061, respectively. Under this condition, a significant difference in the allele frequency between 2,610 case and 2,502 control subjects can be detected with a power >99.6%.

SNP typing. Taqman analysis was used for typing SNP −420, as previously described (17,21). When required, PCR direct sequencing was performed, as described previously (4,22).

Statistical analysis. To analyze differences in SNP −420 frequencies among ages, trend testing using 10-year age intervals was used. Student's t test, ANOVA, or χ^2 test was used where indicated.

RESULTS

We analyzed *RETN* SNP −420 in 2,610 type 2 diabetic case and 2,502 control subjects recruited from six different prefectures in Japan. SNP −420 was in Hardy-Weinberg equilibrium in both case and control subjects. Neither the allele nor the genotype was associated with type 2 diabetes (Table 1).

Since we previously reported that the onset of type 2 diabetes was earlier in subjects with the G/G genotype (4), we examined the allele frequencies and genotype distribution of SNP −420 as a function of subject age. A trend test for 10-year intervals revealed that the G-allele frequency differed significantly among age grades in type 2 diabetic subjects ($P = 0.022$); the G-allele appears to be more frequent in younger type 2 diabetic subjects, especially those aged <40 years, although the increasing trend was not linear ($P = 0.458$) (Fig. 1). In contrast, this increase was not evident in control subjects.

The trend test also revealed that the frequency of the G/G genotype differed significantly among age grades in type 2 diabetic subjects ($P = 0.037$). The G/G genotype also appears to be more frequent in younger type 2 diabetic subjects, especially those below the age of 40 years, although the increasing trend was not linear ($P = 0.265$) (Fig. 2). In constrast, no difference was found in the frequency of the G/G genotype among age grades in control subjects ($P = 0.440$). There appeared to be no differences between male and female subjects (data not shown). Therefore, in type 2 diabetes, the frequency of both the G-allele and the G/G genotype appears to be higher in younger subjects.

Since the G-allele and G/G genotype frequency appear to be high in younger type 2 diabetic subjects, especially those aged <40 years, we compared the allele and genotype frequencies of SNP −420 between type 2 diabetic subjects aged <40 years and those aged ≥40 years (Table 2). The frequencies of either the G-allele or the G/G genotype were higher in the younger group (G-allele for younger group 43.0% vs. older group 33.0%, $P = 0.008$; odds ratio [OR] of G/G to C/C 2.47, $P = 0.003$). When both case and control subjects aged <40 years were analyzed, the frequencies of both the G-allele and the G/G genotype were higher in type 2 diabetic subjects (G-allele in type 2 diabetic subjects 43.0% vs. control subjects 33.3%, $P = 0.016$; OR of G/G to C/C 2.28, $P = 0.012$). Therefore, the G/G genotype at SNP −420 appeared to be associated with type 2 diabetes in younger subjects.

Finally, to examine the relation between SNP −420 and the age of type 2 diabetes onset, we added 237 type 2 diabetic subjects with onset age ≤40 years. To adjust the effect of aging on the increasing frequency of the G-allele, we analyzed a total of 2,430 type 2 diabetic subjects with age of onset <60 years. The trend test revealed that G-allele and G/G genotype had an increasing linear trend as the age grade of type 2 diabetes onset became younger ($P = 0.0492$ and $P = 0.0379$, respectively).

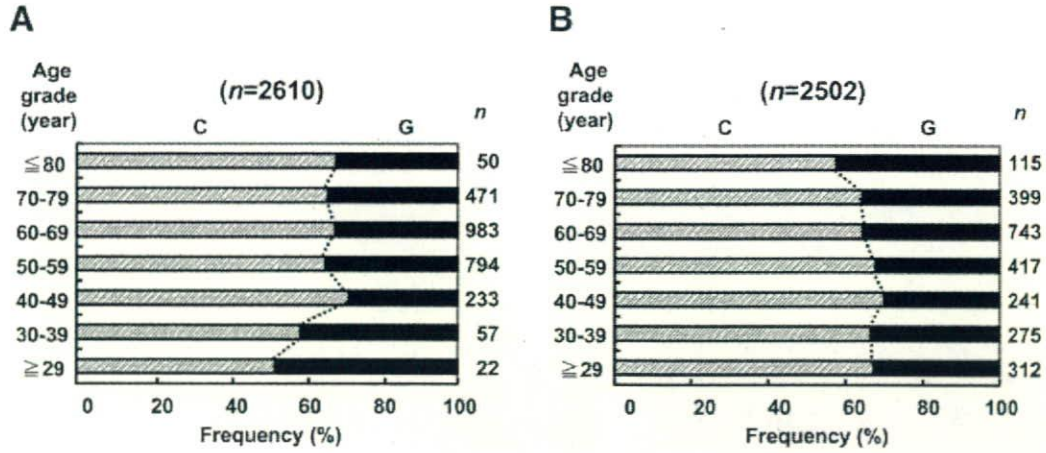

FIG. 1. The frequency of the G-allele of SNP −420 appears to be increased in younger type 2 diabetic subjects and showed an increasing linear trend in older control subjects. The allele frequencies of resistin SNP −420 stratified for 10-year age intervals for type 2 diabetic case (*A*) and control (*B*) subjects are shown. A trend test revealed that the frequency of the G-allele differed among age grades in type 2 diabetic subjects (P = 0.022), although the trend was not linear (P = 0.458). In control subjects, the frequency of the G-allele showed an increasing linear trend with increase in age (P = 0.008).

DISCUSSION

We report here that the G/G genotype at SNP −420 was associated with type 2 diabetes in younger subjects but not in total subjects by analyzing 2,610 type 2 diabetic case and 2,502 control subjects. Differences in G-allele frequencies among age grades in case and control subjects—namely, an increasing linear trend in control subjects in older grades—and an apparent increase in type 2 diabetic cases aged <40 years could result in no association between the SNP −420 genotype and type 2 diabetes in the total subjects. The association of SNP −420 with type 2 diabetes has been controversial, suggesting that a variety of factors could affect the results (4,13,14,16). This discrepancy may be resolved by considering age grades and increasing the number of samples, as suggested by the present study.

We have shown that the G/G genotype frequency was increased in younger type 2 diabetic subjects, in whom genetic factors are thought to have stronger effects on disease susceptibility. Conversely, this finding means that the G/G genotype frequency was decreased with increasing age. It is possible that resistin may become less of a significant risk factor as age increases or that type 2 diabetic patients with the G/G genotype may not live longer. It should be noted that P values observed were marginal and that the sample size, especially that of type 2 diabetic subjects with younger age of onset, was limited in this study. A larger number of samples should be analyzed for replication. When stratified by seven grades (2-kg/m^2 intervals) of BMI, no apparent linear trend of G-allele or G/G genotype was observed in control or type 2 diabetic subjects (data not shown). This supports that the trends in the age stratification are relevant although the effect of possible heterogeneity among areas cannot be completely excluded.

In contrast to type 2 diabetic subjects, a trend test revealed that in control subjects, the G-allele frequency had an increasing linear trend as the age grade became

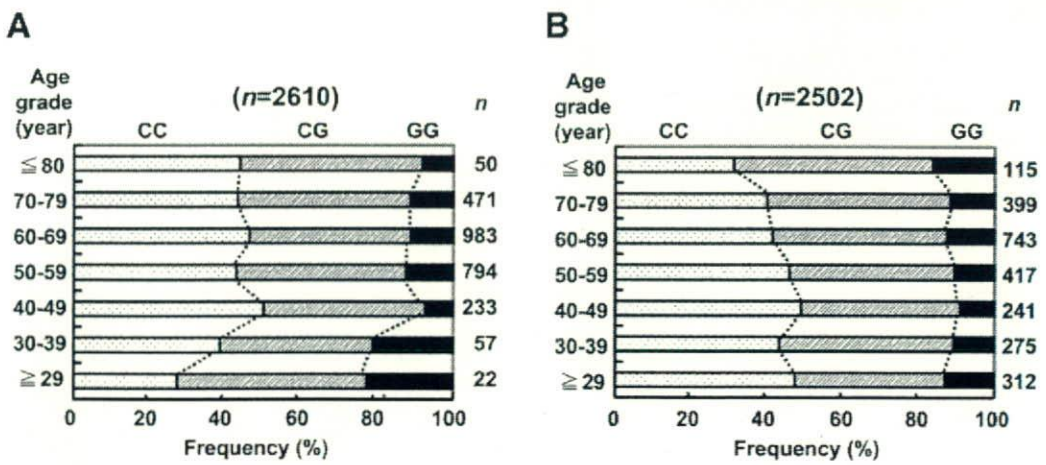

FIG. 2. The frequency of G/G genotype of SNP −420 appears to be increased in younger type 2 diabetic subjects, whereas that of C/G genotype showed an increasing linear trend in older control subjects. The genotype frequencies of resistin SNP −420 stratified by 10-year age intervals for type 2 diabetic case (*A*) and control (*B*) subjects are shown. A trend test revealed that the frequency of the G/G genotype differed among age grades in type 2 diabetic subjects (P = 0.037), though the trend was not linear (P = 0.265). In control subjects, the frequency of the G/G genotype did not differ among age grades (P = 0.440). The frequency of the C/G genotype showed an increasing linear trend with an increase in age in control subjects (P = 0.010), whereas that of the C/C genotype showed an decreasing linear trend (P = 0.002).

TABLE 2
G/G genotype at SNP −420 was associated with type 2 diabetes in younger subjects

Comparison between type 2 diabetic subjects aged <40 years (n = 79) with those aged ≥40 years (n = 2,531)						
Genotype or allele	<40 years old	≥40 years old	Comparison	χ^2	P	OR (95% CI)
CC	28	1,141	CC/CG/GG	8.96	0.011	—
CG	34	1,110	GG vs. CC	8.82	0.003	2.47 (1.34–4.58)
GG	17	280	CG vs. CC	0.74	0.390	1.25 (0.75–2.07)
			GG vs. CG	5.23	0.022	1.98 (1.09–3.60)
			GG + CG vs. CC	2.88	0.090	1.50 (0.94–2.39)
			GG vs. CG + CC	8.31	0.004	2.20 (1.27–3.82)
G-allele	68 (43.0)	1,670 (33.0)	G- vs. C-allele	6.96	0.008	—
Comparison between type 2 diabetic (n = 79) and control (n = 587) subjects aged <40 years						
Genotype or allele	Type 2 diabetic subjects	Control subjects	Comparison	χ^2	P	OR (95% CI)
CC	28	267	CC/CG/GG	6.27	0.044	—
CG	34	249	GG vs. CC	6.31	0.012	2.28 (1.18–4.40)
GG	17	71	CG vs. CC	0.96	0.327	1.30 (0.77–2.21)
			GG vs. CG	3.02	0.082	1.75 (0.93–3.32)
			GG + CG vs. CC	2.85	0.092	1.52 (0.93–2.48)
			GG vs. CG + CC	5.39	0.020	1.99 (1.10–3.60)
G-allele	68 (43.0)	391 (33.3)	G- vs. C-allele	5.84	0.016	—

Data are n or n (%) unless otherwise indicated. χ^2 test was used for statistical analysis. ORs calculated by defining the G-allele as a susceptibility allele.

older (P = 0.008) (Figs. 1*B* and 2*B*). The C/G genotype showed an increasing linear trend in older age grades (P = 0.010), whereas the C/C genotype showed a decreasing linear trend (P = 0.002). There appeared to be no sex differences (data not shown). These findings suggest that *RETN* may be a longevity gene like adiponectin (23) under certain conditions. We previously reported that serum resistin levels were highest in subjects with G/G genotype, followed by C/G and C/C (4,17). Therefore, moderately elevated serum resistin levels in C/G genotype, by reducing insulin signaling, may be beneficial for a longer life in nondiabetic control subjects. The lower serum resistin levels in C/C genotype may not be sufficient to have this effect. In fact, mutations in the insulin receptor homologous gene are known to result in longevity in *elegans* and *Drosophila* (24,25).

Recently, we reported that plasma resistin was correlated with insulin resistance in 2,078 subjects in the Japanese general population (21). Plasma resistin was highest in subjects with the G/G genotype of SNP −420, followed by C/G and C/C. The effect of SNP −420 on plasma resistin was independent of age, sex, and BMI. The 26% of total variance of plasma resistin could be explained by SNP −420, suggesting that not only SNP −420 but also other genetic and environmental factors could affect plasma resistin levels. The direct association between type 2 diabetes and SNP −420 may be more difficult to detect.

In summary, we analyzed *RETN* SNP −420 in 2,610 type 2 diabetic case and 2,502 control subjects. Although SNP −420 was not associated with type 2 diabetes when analyzed without considering subject age, the G/G genotype frequencies appear to be higher in younger subjects with type 2 diabetes. When 237 type 2 diabetic subjects with age of onset ≤40 years were added, in a total of 2,430 type 2 diabetic subjects with age of onset <60 years, the G/G genotype had an increasing linear trend as the age grade of type 2 diabetes onset became younger. Therefore, the G/G genotype frequency was increased in younger type 2 diabetic subjects. In contrast, the C/G genotype showed an increasing linear trend as the age grade became older in control subjects. It is not clear how resistin induces type 2 diabetes in younger subjects or whether it is beneficial for longer life. Further studies will be required to clarify these points.

ACKNOWLEDGMENTS

This work was supported by Grants for Scientific Research from the Ministry of Education, Culture, Science, Sports and Technology of Japan and by grants from Ehime University, Ehime, Japan. We thank our colleagues for collecting clinical data and samples, T. Takasuka and A. Murakami for technical assistance, and Drs. Nishida, Hashiramoto, Takata, Murase, and Nishimiya for suggestions.

REFERENCES

1. DeFronzo RA, Bonadonna RC, Ferrannini E: Pathogenesis of NIDDM: a balanced overview. *Diabetes Care* 15:318–368, 1992
2. McCarthy MI: Progress in defining the molecular basis of type 2 diabetes mellitus through susceptibility-gene identification. *Hum Mol Genet* 13: R33–R41, 2004
3. McCarthy MI, Zeggini E: Genetics of type 2 diabetes. *Curr Diab Rep* 6:147–154, 2006
4. Osawa H, Yamada K, Onuma H, Murakami A, Ochi M, Kawata H, Nishimiya T, Niiya T, Shimizu I, Nishida W, Hashiramoto M, Kanatsuka A, Fujii Y, Ohashi J, Makino H: The G/G genotype of a resistin single-nucleotide polymorphism at -420 increases type 2 diabetes mellitus susceptibility by inducing promoter activity through specific binding of Sp1/3. *Am J Hum Genet* 75:678–686, 2004
5. Steppan C, Lazar M: The current biology of resistin. *J Intern Med* 255:439–447, 2004
6. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA: The hormone resistin links obesity to diabetes. *Nature* 409:307–312, 2001
7. Rangwala S, Rich A, Rhoades B, Shapiro J, Obici S, Rossetti L, Lazar M: Abnormal glucose homeostasis due to chronic hyperresistinemia. *Diabetes* 53:1937–1941, 2004
8. Banerjee R, Rangwala S, Shapiro J, Rich A, Rhoades B, Qi Y, Wang J, Rajala

M, Pocai A, Scherer P, Steppan C, Ahima R, Obici S, Rossetti L, Lazar M: Regulation of fasted blood glucose by resistin. *Science* 303:1195–1198, 2004
9. Patel L, Buckels A, Kinghorn I, Murdock P, Holbrook J, Plumpton C, Macphee C, Smith S: Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. *Biochem Biophys Res Commun* 300:472–476, 2003
10. Savage D, Sewter C, Klenk E, Segal D, Vidal-Puig A, Considine R, O'Rahilly S: Resistin / Fizz3 expression in relation to obesity and peroxisome proliferator–activated receptor-γ action in humans. *Diabetes* 50:2199–2202, 2001
11. Weisberg S, McCann D, Desai M, Rosenbaum M, Leibel R, Ferrante AJ: Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 112:1796–1808, 2003
12. Xu H, Barnes G, Yang Q, Tan G, Yang D, Chou C, Sole J, Nichols A, Ross J, Tartaglia L, Chen H: Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 112:1821–1830, 2003
13. Cho Y, Youn B, Chung S, Kim K, Lee H, Yu K, Park H, Shin H, Park K: Common genetic polymorphisms in the promoter of resistin gene are major determinants of plasma resistin concentrations in humans. *Diabetologia* 47:559–565, 2004
14. Engert JC, Vohl MC, Williams SM, Lepage P, Loredo-Osti JC, Faith J, Dore C, Renaud Y, Burtt NP, Villeneuve A, Hirschhorn JN, Altshuler D, Groop LC, Despres JP, Gaudet D, Hudson TJ: 5′ flanking variants of resistin are associated with obesity. *Diabetes* 51:1629–1634, 2002
15. McTernan P, Fisher F, Valsamakis G, Chetty R, Harte A, McTernan C, Clark P, Smith S, Barnett A, Kumar S: Resistin and type 2 diabetes: regulation of resistin expression by insulin and rosiglitazone and the effects of recombinant resistin on lipid and glucose metabolism in human differentiated adipocytes. *J Clin Endocrinol Metab* 88:6098–6106, 2003
16. Smith S, Bai F, Charbonneau C, Janderova L, Argyropoulos G: A promoter genotype and oxidative stress potentially link resistin to human insulin resistance. *Diabetes* 52:1611–1618, 2003
17. Osawa H, Onuma H, Ochi M, Murakami A, Yamauchi J, Takasuka T, Tanabe F, Shimizu I, Kato K, Nishida W, Yamada K, Tabara Y, Yasukawa M, Fujii Y, Ohashi J, Miki T, Makino H: Resistin SNP −420 determines its monocyte mRNA and serum levels inducing type 2 diabetes. *Biochem Biophys Res Commun* 335:596–602, 2005
18. Matsumoto H: Characteristics of Mongoloid and neighboring populations based on the genetic markers of human immunoglobulins. *Hum Genet* 80:207–218, 1988
19. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 26 (Suppl. 1):S5–S20, 2003
20. Ohashi J, Yamamoto S, Tsuchiya N, Hatta Y, Komata T, Matsushita M, Tokunaga K: Comparison of statistical power between 2 * 2 allele frequency and allele positivity tables in case-control studies of complex disease genes. *Ann Intern Med* 65:197–206, 2001
21. Osawa H, Tabara Y, Kawamoto R, Ohashi J, Ochi M, Onuma H, Nishida W, Yamada K, Nakura J, Kohara K, Miki T, Makino H: Plasma resistin, associated with single nucleotide polymorphism −420, is correlated with insulin resistance, lower HDL, and high-sensitivity C-reactive protein in the Japanese general population. *Diabetes Care* 30:1501–1506, 2007
22. Osawa H, Onuma H, Murakami A, Ochi M, Nishimiya T, Kato K, Shimizu I, Fujii Y, Ohashi J, Makino H: Systematic search for single nucleotide polymorphisms in the resistin gene: the absence of evidence for the association of three identified single nucleotide polymorphisms with Japanese type 2 diabetes. *Diabetes* 51:863–866, 2002
23. Bik W, Baranowska-Bik A, Wolinska-Witort E, Martynska L, Chmielowska M, Szybinska A, Broczek K, Baranowska B: The relationship between adiponectin levels and metabolic status in centenarian, early elderly, young and obese women. *Neuro Endocrinol Lett* 27:493–500, 2006
24. Kimura KD, Tissenbaum HA, Liu Y, Ruvkun G: daf-2, an insulin receptor-like gene that regulates longevity and diapause in Caenorhabditis elegans. *Science* 277:942–946, 1997
25. Tatar M, Kopelman A, Epstein D, Tu MP, Yin CM, Garofalo RS: A mutant Drosophila insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* 292:107–110, 2001

Diabetologia (2007) 50:747–751
DOI 10.1007/s00125-006-0588-6

SHORT COMMUNICATION

A genetic variation of the transcription factor 7-like 2 gene is associated with risk of type 2 diabetes in the Japanese population

M. Horikoshi · K. Hara · C. Ito · R. Nagai · P. Froguel · T. Kadowaki

Received: 9 August 2006 / Accepted: 4 December 2006 / Published online: 24 January 2007

Abstract
Aims/hypothesis It has been suggested that transcription factor 7-like 2 protein (TCF7L2) plays an important role in glucose metabolism by regulating the production level of glucagon-like peptide-1, a hormone which modifies glucose-dependent insulin secretion. Recently, variants of *TCF7L2* gene were reported to confer an increased risk of type 2 diabetes in three different samples from European and European-origin populations. We studied whether the single nucleotide polymorphisms (SNPs) in *TCF7L2* were associated with type 2 diabetes in samples from a Japanese population.
Methods Five SNPs were genotyped in three different sample sets. Association with type 2 diabetes was investigated in each, as well as in combined sample sets.
Results The SNP rs7903146 was nominally associated with type 2 diabetes in the initial (p=0.08) and two replication sample sets (p=0.05 and 0.06). For the combined sample set, in which we successfully genotyped 1,174 type 2 diabetes patients and 823 control subjects, rs7903146 showed a significant association with type 2 diabetes (odds ratio=1.69 [95% CI 1.21–2.36], p=0.002) with the same direction as the previous reports in samples from European and European-origin populations. SNPs rs7903146 and rs7901695 were in complete linkage disequilibrium. The rest of the five SNPs (rs7895340, rs11196205 and rs12255372) did not show any significant associations with type 2 diabetes.
Conclusions/interpretation The consistent association between rs7903146 in *TCF7L2* and type 2 diabetes in different ethnic groups, including the Japanese population, suggests that *TCF7L2* is a common susceptibility gene for type 2 diabetes.

Keywords Association · Susceptibility gene · Type 2 diabetes

Abbreviations

GLP-1	glucagon-like peptide-1
HOMA	homeostasis model assessment
LD	linkage disequilibrium
OR	odds ratio
PAR	population attributable risk
SNP	single nucleotide polymorphism
TCF7L2	transcription factor 7-like 2

M. Horikoshi and K. Hara contributed equally to the present study.

Electronic supplementary material The online version of this article (doi:10.1007/s00125-006-0588-6) contains supplementary material, which is available to authorised users.

M. Horikoshi · K. Hara · T. Kadowaki (✉)
Department of Metabolic Diseases, Graduate School of Medicine, University of Tokyo,
Hongo 7-3-1, Bunkyo-ku,
Tokyo 113-8655, Japan
e-mail: kadowaki-3im@h.u-tokyo.ac.jp

M. Horikoshi · K. Hara · T. Kadowaki
CREST, Japan Science and Technology Corporation (JST),
Tokyo, Japan

C. Ito
Medical Court Life Care Clinic,
Hiroshima, Japan

R. Nagai
Department of Cardiovascular Medicine,
Graduate School of Medicine, University of Tokyo,
Tokyo, Japan

P. Froguel
Genomics and Molecular Physiology of Metabolic Diseases,
CNRS UMR,
8090 Lille Cedex, France

Introduction

Transcription factor 7-like 2 protein (TCF7L2) regulates the production level of proglucagon, which is the precursor of the insulinotropic hormone glucagon-like peptide 1 (GLP-1) in enteroendocrine cells [1]. GLP-1 exerts critical effects on blood glucose homeostasis by increasing insulin secretion. TCF7L2 also has an essential role in the developmental and growth regulatory mechanisms of intestinal epithelial cells which secrete GLP-1, because *TCF7L2*-deficient mice lack an intestinal epithelial stem cell compartment [2]. TCF7L2 could influence susceptibility to type 2 diabetes by altering levels of GLP-1 and/or other hormones. Moreover, *TCF7L2* is located in a chromosomal region that has been reported to be linked to type 2 diabetes in the Icelandic population [3]. Therefore, *TCF7L2* is a plausible candidate gene for type 2 diabetes. Recently, the Icelandic group reported that genetic variations located in introns of *TCF7L2* were significantly associated with type 2 diabetes in samples from Icelandic, Danish and US populations [4].

In this study, we investigated whether the previously demonstrated genetic variations shown to be strongly associated with type 2 diabetes in the samples from European and European-origin populations are also associated with type 2 diabetes in samples from a Japanese population.

Subjects and methods

Subjects We performed association studies in three different sample sets (Table 1). In the initial and replication sample sets, diabetic patients were randomly recruited from among those attending the outpatient clinic of the Department of Metabolic Diseases, Graduate School of Medicine, University of Tokyo (Tokyo, Japan), and the non-diabetic subjects from among those undergoing annual health check-ups at the Hiroshima Atomic Bomb Casualty Council Health Management Center (Hiroshima, Japan). Another unrelated 356 diabetic patients and 192 control subjects were recruited from the same region and same facility in Hiroshima to avoid bias due to population stratification. The inclusion criteria for non-diabetic subjects were as follows: (1) >60 years of age; (2) HbA_{1c} values <5.8%; and (3) no family history of type 2 diabetes in first- and second-degree relatives. Diabetes was diagnosed in accordance with WHO criteria [5]. All participants gave informed consent, and the Ethics Committee of the University of Tokyo approved this study.

Single nucleotide polymorphism genotyping We genotyped all five *TCF7L2* single nucleotide polymorphisms (SNPs) previously described by Grant et al. [4]. rs7901695 and rs7895340 were genotyped by direct sequencing, performed with a BigDye terminator (Applied Biosystems, Foster City, CA, USA) and resolved using an ABI 3700 automated DNA sequencer (Applied Biosystems). rs7903146, rs11196205 and rs12255372 were genotyped using Taqman SNP Genotyping Assays by means of an ABI 7900HT (Applied Biosystems) according to the manufacturer's protocol. Genotyping success rates were 99% (rs7901695), 98% (rs7903146), 94% (rs7895340), 99% (rs11196205) and 99% (rs12255372). Concordance rate, based on duplicate comparisons in 192 control subjects and 192 type 2 diabetes, was 100%. All the SNPs were in accordance with Hardy–Weinberg equilibrium in type 2 diabetes subjects ($p>0.17$), control subjects ($p>0.5$) and the whole subject group ($p>0.13$).

Biological measurements Insulin resistance and beta cell function were quantified using homeostasis model assessment (HOMA-IR and HOMA-beta, respectively); HOMA-IR=(fasting insulin [pmol/l])×glucose [mmol/l])/22.5×6 and HOMA-beta=(fasting insulin [pmol/l])×20)/(glucose [mmol/l]−3.5)×6 as described elsewhere [6] (original equation modified to incorporate SI units, with mU/l converted to pmol/l). Data were expressed as means ± SD.

Table 1 Clinical characteristics (means ± SD) of the subjects

	Initial		Replication		Hiroshima		All subjects	
	Diabetes	Control	Diabetes	Control	Diabetes	Control	Diabetes	Control
n	192	272	657	360	356	192	1,205	824
Male	123	129	421	141	217	93	761	363
Female	69	143	236	219	139	99	444	461
Age at onset of diabetes (years)	48.4±11.2	NA	49.8±10.9	NA	57.5±11.0	NA	51.9±11.6	NA
Age at examination (years)	62.8±9.6	68.6±6.7	63.2±9.4	70.5±6.8	70.6±7.1	68.6±6.7	65.3±9.4	69.4±6.8
BMI (kg/m^2)	23.9±3.9	24.0±3.8	24.4±3.9	23.6±3.5	24.0±3.3	23.8±4.0	24.2±3.7	23.8±3.7
Fasting glucose (mmol/l)	8.9±3.0	5.2±0.5	8.9±3.0	5.0±0.8	8.0±3.2	5.2±0.9	8.6±3.1	5.1±0.7
HbA_{1c} (%)	7.4±1.4	5.2±0.3	7.6±1.7	5.2±0.2	6.9±1.2	5.2±0.2	7.4±1.6	5.2±0.2

NA Not applicable

Statistical analysis The proportions of genotypes or alleles were compared between type 2 diabetic and non-diabetic subjects using a multiple logistic regression analysis adjusted for age, sex and BMI. Differences in HOMA according to genotypes were determined by analysis of covariates in non-diabetic subjects, after adjustment for age, sex and BMI. The statistical analyses were performed using JMP for Windows version 4.00 software (SAS Institute, Cary, NC, USA). To examine the pairwise linkage disequilibrium (LD) structure, r^2 between the SNPs were estimated via the method of maximum likelihood from two-locus genotype data using the expectation–maximisation algorithm under the assumption of Hardy–Weinberg equilibrium. The calculations were performed with SNPAlyze v3.2 Pro software (Dynacom, Yokohama, Japan). We considered $p<0.05$ to be significant. The odds ratio (OR) was assessed by counting the number of risk alleles for each individual, and we used the number of risk alleles to predict case/control status using logistic regression. Population attributable risk (PAR) was calculated as $PAR=(p[OR-1])/(1+p[OR-1])$, where p is the prevalence of subjects with the risk allele.

Results

Genotype and allele frequencies of the five SNPs in *TCF7L2* are shown in Table 2. Genotype frequency of rs7903146 was nominally associated with type 2 diabetes in each sample set ($p=0.08$, 0.05, 0.06; initial, replication and Hiroshima sample sets, respectively). However, when all the samples were combined, both genotype and minor allele frequencies were significantly associated with type 2 diabetes (OR 1.69 [95% CI 1.21–2.36], $p=0.0075$ and $p=0.002$; genotype and minor allele frequencies, respectively). This significant association did not change when adjustment for BMI was omitted from the multiple logistic regression analysis (data not shown). The minor allele frequency of rs7903146 in the samples from a Japanese population (0.03–0.05) was substantially smaller than that of the previously reported three samples from European and European-origin populations, in which minor allele frequencies ranged from 0.27 to 0.39. Association between rs7903146 and type 2 diabetes in a dominant model was also examined, as its minor allele frequency was very low, but did not reach significance in two of our three sample sets. However, when all the samples were combined, we could confirm the significantly increased risk of type 2 diabetes in subjects with CT or TT (10.5 vs 6.4%, type 2 diabetes vs controls, respectively; OR 1.75 [95% CI 1.23–2.48], $p=0.0018$).

We found a significant interaction between SNP rs7903146 and BMI ($p=0.031$) in the logistic regression analysis, suggesting that the effect of SNP rs7903146 on the risk of type 2 diabetes was different according to BMI. When we restricted the subjects to those with BMI lower than the median (BMI<23.8 and <23.5 kg/m^2, for type 2 diabetes patients and control subjects, respectively), rs7903146 showed a higher OR (2.02 [95% CI 1.28–3.21], $p=0.0027$; Electronic supplementary Table 1). The association was negative in subjects with BMI higher than the median (OR 1.32 [95% CI 0.81–2.17], $p=0.27$).

rs7901695 showed a significant association with type 2 diabetes in the initial sample set (minor allele frequency: diabetes/control; 0.07/0.04, $p=0.04$), and because it was in complete LD ($r^2=1.0$; Electronic supplementary Fig. 1) with rs7903146, we did not conduct further genotyping in the replication and Hiroshima sample sets. There were no significant differences in genotype or allele frequencies between type 2 diabetes patients and control subjects regarding rs7895340, rs11196205 and rs12255372 (Table 2).

We tested the SNP rs7903146 for quantitative trait association in non-diabetic subjects. rs7903146 did not show any association with age, sex, BMI and other clinical parameters related to type 2 diabetes such as HbA_{1c}, fasting glucose, fasting insulin, HOMA-IR (CC vs CT/TT; 1.83±1.2 vs 1.65±0.8, $p=0.25$), and HOMA-beta (CC vs CT/TT; 100.0±65.7 vs 97.6±49.8, $p=0.79$) in the non-diabetic subjects.

Discussion

In this study, we found a significant association in samples from a Japanese population between the variation in *TCF7L2* and type 2 diabetes, an association similar to that previously reported in samples from European and European-origin populations [4, 7–13]. It is noteworthy that the association between the SNP in *TCF7L2* and type 2 diabetes has consistently been observed in different ethnic groups [14, 15], which supports the reliability of both previous studies as well as our present study. The mechanism of action of TCF7L2 in glucose metabolism and the pathogenesis of type 2 diabetes has yet to be elucidated, but it is possible that TCF7L2 has a role in regulating glucose-sensitive insulin secretion from beta cells. The prevalence of type 2 diabetes in the Japanese population is as high as in the USA [16], although the prevalence of obesity is much lower than that seen in Western countries [17]. One of the possible explanations is that fewer Japanese than European subjects are able to secrete enough insulin to compensate adequately for insulin resistance due to obesity [18]. Therefore, it is important to clarify the genetic components of susceptibility to insulin deficiency. Based on the present results, subjects having an at-risk allele account for 4% of the population, and the corresponding PAR is 3%, a value much lower than that in samples from European and European-origin populations

Table 2 Genotype and allele frequencies (*n* [%]) of *TCF7L2* SNPs

	Initial			Replication			Hiroshima			All subjects			Minor allele frequency		OR (95% CI)[c]
	Diabetes	Control	*p* value[a]	Diabetes	Control	*p* value[a]	Diabetes	Control	*p* value[a]	Diabetes	Control	*p* value[a]	Diabetes/control	*p* value[b]	
rs7901695[d]															
CC	165 (87)	251 (92.3)													
CT	22 (12)	21 (7.7)													
TT	2 (1)	0 (0)	0.08												
rs7903146															
CC	165 (87)	251 (92.3)		584 (89.8)	338 (94)		302 (90)	181 (95)		1,051 (89.5)	770 (93.6)				
CT	22 (12)	21 (7.7)		64 (9.8)	20 (5.5)		33 (10)	10 (5)		119 (10.2)	51 (6.2)		0.05/0.03	0.002	
TT	2 (1)	0 (0)	0.08	2 (0.4)	2 (0.5)	0.05	0 (0)	0 (0)	0.06	4 (0.3)	2 (0.2)	0.0075			1.69 (1.21–2.36)
rs7895340															
GG	148 (85)	226 (90.4)		559 (87.6)	306 (90)		308 (91.7)	162 (90.5)		1,015 (88.4)	694 (90.2)				
GA	23 (13)	23 (9.2)		75 (11.8)	32 (9.4)		27 (8)	17 (9.5)		125 (10.9)	72 (9.4)		0.06/0.05	0.16	
AA	3 (2)	1 (0.4)	0.15	4 (0.6)	2 (0.6)	0.53	1 (0.3)	0 (0)	0.65	8 (0.7)	3 (0.4)	0.37			1.18 (0.88–1.58)
rs11196205															
GG	161 (83.9)	244 (90)		578 (88)	319 (88.6)		310 (89)	172 (90.7)		1,049 (87.6)	739 (89.6)				
GC	28 (14.6)	27 (10)		76 (11.6)	38 (10.6)		35 (10)	18 (9.3)		139 (11.6)	83 (10)		0.06/0.05	0.13	
CC	3 (1.5)	0 (0)	0.03	3 (0.4)	3 (0.8)	0.67	3 (1)	0 (0)	0.40	9 (0.8)	3 (0.4)	0.29			1.21 (0.92–1.56)
rs12255372															
GG	175 (93)	254 (94)		615 (93.6)	343 (95.3)		330 (95.1)	185 (96.4)		1,120 (93.7)	782 (95.0)				
GT	16 (7)	17 (6)		41 (6.2)	16 (4.4)		16 (4.6)	7 (3.6)		73 (6.1)	40 (4.9)		0.03/0.02	0.21	
TT	0 (0)	0 (0)	0.46	1 (0.2)	1 (0.3)	0.43	1 (0.3)	0 (0)	0.64	2 (0.2)	1 (0.1)	0.44			1.21 (0.82–1.81)

[a] *p* values are based on genotype frequencies.
[b] *p* values are based on allele frequencies.
[c] ORs were calculated using an additive genetic model that in logistic regression is multiplicative on the OR scale. OR for each SNP was adjusted simultaneously for age, sex and BMI.
[d] Replication and Hiroshima sample sets were not genotyped owing to the complete LD between rs7901695 and rs7903146.

(21%) [4]. It is possible that *TCF7L2* plays a substantial role in genetic susceptibility to insulin deficiency in the Japanese population. Florez et al. [19] reported that *TCF7L2* polymorphisms were associated with increased risk of developing type 2 diabetes in samples from a population of European origins. In that study, associations between *TCF7L2* polymorphisms and type 2 diabetes in other ethnic groups including Asians were also investigated. However, no significant associations were identified, possibly due to the small sample size. The present study provides important information suggesting *TCF7L2* is a type 2 diabetes susceptibility gene common to various ethnic groups including Japanese.

Acknowledgement This work was supported by a Grant-in-aid (to T. Kadowaki) from the Organization for Pharmaceutical Safety and Research and by a Grant-in-aid for the 21st Century COE Program (to R. Nagai). We thank Y. Miyama for her technical assistance.

Duality of interest There is no duality of interest.

References

1. Yi F, Brubaker PL, Jin T (2005) TCF-4 mediates cell type-specific regulation of proglucagon gene expression by beta-catenin and glycogen synthase kinase-3beta. J Biol Chem 280:1457–1464
2. Korinek V, Barker N, Moerer P et al (1998) Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf4. Nat Genet 19:379–383
3. Reynisdottir I, Thorleifsson G, Benediktsson R et al (2003) Localization of a susceptibility gene for type 2 diabetes to chromosome 5q34–q35.2. Am J Hum Genet 73:323–325
4. Grant SF, Thorleifsson G, Reynisdottir I et al (2006) Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. Nat Genet 38:320–322
5. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (1997) Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 20:1183–1197
6. Matthews DR, Hosker JP, Rudenski AS et al (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
7. Groves CJ, Zeggini E, Minton J (2006) Association analysis of 6,736 U.K. subjects provides replication and confirms TCF7L2 as a type 2 diabetes susceptibility gene with a substantial effect on individual risk. Diabetes 55:2640–2644
8. Zhang C, Qi L, Hunter DJ (2006) Variant of transcription factor 7-like 2 (TCF7L2) gene and the risk of type 2 diabetes in large cohorts of U.S. women and men. Diabetes 55:2645–2648
9. Scott LJ, Bonnycastle LL, Willer CJ et al (2006) Association of transcription factor 7-like 2 (TCF7L2) variants with type 2 diabetes in a Finnish sample. Diabetes 55:2649–2653
10. Damcott CM, Pollin TI, Reinhart LJ et al (2006) Polymorphisms in the transcription factor 7-like 2 (TCF7L2) gene are associated with type 2 diabetes in the Amish: replication and evidence for a role in both insulin secretion and insulin resistance. Diabetes 55:2654–2659
11. Saxena R, Gianniny L, Burtt NP et al (2006) Common single nucleotide polymorphisms in TCF7L2 are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in nondiabetic individuals. Diabetes 55:2890–2895
12. Cauchi S, Meyre D, Dina C et al (2006) Transcription factor TCF7L2 genetic study in the French population: expression in human beta-cells and adipose tissue and strong association with type 2 diabetes. Diabetes 55:2903–2908
13. van Vliet-Ostaptchouk JV, Shiri-Sverdlov R, Zhernakova A et al (2006) Association of variants of transcription factor 7-like 2 (TCF7L2) with susceptibility to type 2 diabetes in the Dutch Breda cohort. Diabetologia DOI 10.1007/s00125-006-0477-z
14. Humphries SE, Gable D, Cooper JA et al (2006) Common variants in the TCF7L2 gene and predisposition to type 2 diabetes in UK European Whites, Indian Asians and Afro-Caribbean men and women. J Mol Med 84(Suppl 1):1–10
15. Chandak GR, Janipalli CS, Bhaskar S et al (2006) Common variants in the TCF7L2 gene are strongly associated with type 2 diabetes mellitus in the Indian population. Diabetologia DOI 10.1007/s00125-006-0502-2
16. Wild S, Roglic G, Green A et al (2004) Global prevalence of diabetes. Diabetes Care 27:1047–1053
17. Yach D, Stuckler D, Brownell K (2006) Epidemiologic and economic consequences of the global epidemics of obesity and diabetes. Nat Med 12:62–66
18. Jensen CC, Cnop M, Hull RL et al (2002) Beta-cell function is a major contributor to oral glucose tolerance in high-risk relatives of four ethnic groups in the U.S. Diabetes 51:2170–2178
19. Florez JC, Jablonski KA, Bayley N et al (2006) TCF7L2 polymorphisms and progression to diabetes in the Diabetes Prevention Program. N Engl J Med 355:241–250

Brief Genetics Report

Association Studies of Variants in the Genes Involved in Pancreatic β-Cell Function in Type 2 Diabetes in Japanese Subjects

Norihide Yokoi,[1] Masao Kanamori,[2] Yukio Horikawa,[3] Jun Takeda,[3] Tokio Sanke,[4] Hiroto Furuta,[5] Kishio Nanjo,[5] Hiroyuki Mori,[6] Masato Kasuga,[6] Kazuo Hara,[7] Takashi Kadowaki,[7] Yukio Tanizawa,[8] Yoshitomo Oka,[9] Yukiko Iwami,[10] Hisako Ohgawara,[10] Yuichiro Yamada,[11] Yutaka Seino,[11] Hideki Yano,[12] Nancy J. Cox,[13] and Susumu Seino[1]

Because impaired insulin secretion is characteristic of type 2 diabetes in Asians, including Japanese, the genes involved in pancreatic β-cell function are candidate susceptibility genes for type 2 diabetes. We examined the association of variants in genes encoding several transcription factors (*TCF1*, *TCF2*, *HNF4A*, *ISL1*, *IPF1*, *NEUROG3*, *PAX6*, *NKX2–2*, *NKX6–1*, and *NEUROD1*) and genes encoding the ATP-sensitive K^+ channel subunits Kir6.2 (*KCNJ11*) and SUR1 (*ABCC8*) with type 2 diabetes in a Japanese cohort of 2,834 subjects. The exon 16 −3c/t variant rs1799854 in *ABCC8* showed a significant association ($P = 0.0073$), and variants in several genes showed nominally significant associations ($P < 0.05$) with type 2 diabetes. Although the E23K variant rs5219 in *KCNJ11* showed no association with diabetes in Japanese (for the K allele, odds ratio [OR] 1.08 [95% CI 0.97–1.21], $P = 0.15$), 95% CI around the OR overlaps in meta-analysis of European populations, suggesting that our results are not inconsistent with the previous studies. This is the largest association study so far conducted on these genes in Japanese and provides valuable information for comparison with other ethnic groups. *Diabetes* 55:2379–2386, 2006

Impaired insulin secretion and insulin resistance both contribute to the pathogenesis of type 2 diabetes. The former is a characteristic feature of type 2 diabetes, especially in Asians including Japanese (1), and genes encoding proteins critical in pancreatic β-cell function are therefore particularly good candidate susceptibility genes for type 2 diabetes for this population. Studies of maturity-onset diabetes of the young in humans (2) and knockout mice (3) have shown that mutations of transcription factors required for development, differentiation, and maintenance of the pancreatic β-cells can cause diabetes. Pancreatic β-cell ATP-sensitive K^+ channels (K_{ATP} channels) are crucial in the regulation of insulin secretion by coupling cell metabolism to membrane electrical activity. The pancreatic β-cell K_{ATP} channel comprises two subunits, the inwardly rectifying potassium channel Kir6.2 (*KCNJ11*) and the sulfonylurea receptor SUR1 (*ABCC8*) (4). Mutations in the genes (*ABCC8* and *KCNJ11*) can cause familial persistent hyperinsulinemic hypoglycemia of infancy (5) and permanent neonatal diabetes (6). Several polymorphisms in these genes also have been reported to be associated with type 2 diabetes in populations with distinct ethnic backgrounds (7–20). However, a large-scale association study of these genes has not been performed in type 2 diabetes in the Japanese population. Here, we report on the association of variants in genes encoding various transcription factors and pancreatic β-cell K_{ATP} channel subunits with type 2 diabetes in a large Japanese cohort.

A case-control association study using 1,590 Japanese diabetic subjects and 1,244 nondiabetic control subjects was performed. All subjects were genotyped for 33 variants of 12 genes including transcription factors (*TCF1*, *TCF2*, *HNF4A*, *ISL1*, *IPF1*, *NEUROG3*, *PAX6*, *NKX2–2*, *NKX6–1*, and *NEUROD1*) and β-cell K_{ATP} channel subunits (*KCNJ11* and *ABCC8*) (Table 1).

Results of Hardy-Weinberg equilibrium (HWE) tests are shown in Table 1 of the online appendix (available at http://diabetes.diabetesjournals.org). All genotypes were in HWE, except for departures in cases at *TCF2*_SNP (single nucleotide polymorphism) 5 rs2689, *TCF2*_SNP6

From the [1]Division of Cellular and Molecular Medicine, Kobe University Graduate School of Medicine, Kobe, Japan; the [2]Division of Health and Preventive Medicine, Department of Lifelong Sport, Biwako Seikei Sport College, Shiga, Japan; the [3]Department of Endocrinology, Diabetes and Rheumatology, Division of Bioregulatory Medicine, Gifu University School of Medicine, Gifu, Japan; the [4]Department of Clinical Laboratory Medicine, Wakayama University of Medical Science, Wakayama, Japan; the [5]First Department of Medicine, Wakayama University of Medical Science, Wakayama, Japan; the [6]Department of Clinical Molecular Medicine, Division of Diabetes and Digestive and Kidney Diseases, Kobe University Graduate School of Medicine, Kobe, Japan; the [7]Department of Metabolic Diseases, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; the [8]Division of Molecular Analysis of Human Disorders, Department of Bio-Signal Analysis, Yamaguchi University Graduate School of Medicine, Ube, Japan; the [9]Division of Molecular Metabolism and Diabetes, Tohoku University Graduate School of Medicine, Sendai, Japan; the [10]Division of Cell Replacement and Regenerative Medicine, Medical Research Institute, School of Medicine, Tokyo Women's Medical University, Tokyo, Japan; the [11]Department of Diabetes and Clinical Nutrition, Kyoto University Graduate School of Medicine, Kyoto, Japan; the [12]Department of Internal Medicine, Hikone Municipal Hospital, Shiga, Japan; and the [13]Departments of Medicine and Human Genetics, University of Chicago, Chicago, Illinois.

Address correspondence and reprint requests to Susumu Seino, Division of Cellular and Molecular Medicine, Kobe University Graduate School of Medicine, Chuo-ku, Kobe 650-0017, Japan. E-mail: seino@med.kobe-u.ac.jp.

Received for publication 13 September 2005 and accepted in revised form 22 May 2006.

Additional information for this article can be found in an online appendix at http://diabetes.diabetesjournals.org.

HWE, Hardy-Weinberg equilibrium; K_{ATP} channel, ATP-sensitive K^+ channel; LD, linkage disequilibrium; SNP, single nucleotide polymorphism.

DOI: 10.2337/db05-1203

TABLE 1
Summary of association studies of 33 variants for 12 genes with type 2 diabetes

Number	Locus	HapMap data	Subject	Allele data (frequency) 1	Allele data (frequency) 3	Genotype data (frequency) 1	Genotype data (frequency) 2	Genotype data (frequency) 3	*P* value Allele 2*2	*P* value Genotype 2*3	OR (95% CI)
1	TCF1_SNP1			A	C	A/A	A/C	C/C			
				1,590	1,590						
	rs1169288	JPT	Case	(0.50)	(0.50)	385 (0.24)	820 (0.52)	385 (0.24)	0.4508	0.2388	1.04 (0.94–1.16)
				1,270	1,218						
	I27L		Control	(0.51)	(0.49)	332 (0.27)	606 (0.49)	306 (0.25)			
2	TCF1_SNP2			G	A	G/G	G/A	A/A			
				1,702	1,478						
	rs1169294	none	Case	(0.54)	(0.46)	443 (0.28)	816 (0.51)	331 (0.21)	0.3247	0.1566	1.06 (0.95–1.17)
				1,298	1,190						
	IVS1 −42		Control	(0.52)	(0.48)	348 (0.28)	602 (0.48)	294 (0.24)			
3	TCF1_SNP3			A	T	A/A	A/T	T/T			
					2,698			1,146			
	rs2071190	JPT	Case	482 (0.15)	(0.85)	38 (0.02)	406 (0.26)	(0.72)	0.8925	0.8617	1.01 (0.87–1.17)
					2,115						
	IVS2 −51		Control	373 (0.15)	(0.85)	26 (0.02)	321 (0.26)	897 (0.72)			
4	TCF2_SNP1			G	A	G/G	G/A	A/A			
				2,079	1,101						
	rs757210	JPT	Case	(0.65)	(0.35)	697 (0.44)	685 (0.43)	208 (0.13)	0.4565	0.2695	1.04 (0.93–1.17)
				1,651							
	IVS2 + 2916		Control	(0.66)	837 (0.34)	546 (0.44)	559 (0.45)	139 (0.11)			
5	TCF2_SNP2			A	G	A/A	A/G	G/G			
				1,460	1,720						
	rs757211	none	Case	(0.46)	(0.54)	342 (0.22)	776 (0.49)	472 (0.30)	0.6994	0.5473	1.02 (0.92–1.14)
				1,156	1,332						
	IVS2 + 2953		Control	(0.46)	(0.54)	262 (0.21)	632 (0.51)	350 (0.28)			
6	TCF2_SNP3			G	A	G/G	G/A	A/A			
				2,288							
	rs718960	JPT	Case	(0.72)	892 (0.28)	824 (0.52)	640 (0.40)	126 (0.08)	0.6121	0.8597	1.03 (0.92–1.16)
				1,774							
	IVS4 + 14307		Control	(0.71)	714 (0.29)	632 (0.51)	510 (0.41)	102 (0.08)			
7	TCF2_SNP4			T	A	T/T	T/A	A/A			
				2,823		1,260					
	rs1016991	JPT	Case	(0.89)	357 (0.11)	(0.79)	303 (0.19)	27 (0.02)	0.0105*	0.0399*	1.23 (1.05–1.45)
				2,152							
	IVS8 + 929		Control	(0.87)	336 (0.14)	938 (0.75)	276 (0.22)	30 (0.02)			
8	TCF2_SNP5			A	T	A/A	A/T	T/T			
				1,722	1,458						
	rs2689	JPT	Case	(0.54)	(0.46)	488 (0.31)	746 (0.47)	356 (0.22)	0.5195	0.3582	1.04 (0.93–1.15)
				1,325	1,163						
	+274 TGA		Control	(0.53)	(0.47)	355 (0.29)	615 (0.49)	274 (0.22)			
9	TCF2_SNP6			A	C	A/A	A/C	C/C			
				1,840	1,340						
	rs2688	JPT	Case	(0.58)	(0.42)	552 (0.35)	736 (0.46)	302 (0.19)	0.0563	0.0291*	1.11 (1.00–1.24)

				1,503							
	+444 TGA		Control	(0.60)	985 (0.40)	448 (0.36)	607 (0.49)	189 (0.15)			
10	HNF4A_SNP1			T	C	T/T	T/C	C/C			
				2,277							
	rs717247	JPT	Case	(0.72)	903 (0.28)	811 (0.51)	655 (0.41)	124 (0.08)	0.2071	0.4223	1.08 (0.96–1.22)
				1,820							
	−4229		Control	(0.73)	668 (0.27)	661 (0.53)	498 (0.40)	85 (0.07)			
11	HNF4A_SNP2			A	G	A/A	A/G	G/G			
				1,207	1,973						
	rs736820	none	Case	(0.38)	(0.62)	230 (0.14)	747 (0.47)	613 (0.39)	0.4482	0.6472	1.04 (0.94–1.16)
					1,569						
	IVS1 + 3889		Control	919 (0.37)	(0.63)	176 (0.14)	567 (0.46)	501 (0.40)			
12	HNF4A_SNP3			T	C	T/T	T/C	C/C			
					2,537			1,000			
	rs745975	JPT	Case	643 (0.20)	(0.80)	53 (0.03)	537 (0.34)	(0.63)	0.0470*	0.0769	1.15 (1.00–1.31)
					2,038						
	IVS1 −5		Control	450 (0.18)	(0.82)	39 (0.03)	372 (0.30)	833 (0.67)			
13	ISL1_SNP10			G	A	G/G	G/A	A/A			
				2,842		1,271					
	rs2303750	none	Case	(0.89)	338 (0.11)	(0.80)	300 (0.19)	19 (0.01)	0.5101	0.4453	1.06 (0.90–1.26)
				2,209							
	IVS3 −4		Control	(0.89)	279 (0.11)	987 (0.79)	235 (0.19)	22 (0.02)			
14	ISL1_SNP11			A	G	A/A	A/G	G/G			
				2,466							
	rs2303751	none	Case	(0.78)	714 (0.22)	958 (0.60)	550 (0.35)	82 (0.05)	0.0539	0.1224	1.14 (1.00–1.29)
				1,983							
	P165P		Control	(0.80)	505 (0.20)	787 (0.63)	409 (0.33)	48 (0.04)			
15	IPF1_SNP3			G	T	G/G	G/T	T/T			
				1,688	1,492						
	rs4430606	none	Case	(0.53)	(0.47)	451 (0.28)	786 (0.49)	353 (0.22)	0.1807	0.3405	1.06 (0.90–1.26)
				1,366	1,122						
	IVS1 + 512		Control	(0.55)	(0.45)	371 (0.30)	624 (0.50)	249 (0.20)			
16	IPF1_SNP4			A	C	A/A	A/C	C/C			
				2,527		1,007					
	rs1124607	JPT	Case	(0.79)	653 (0.21)	(0.63)	513 (0.32)	70 (0.04)	0.7609	0.6259	1.02 (0.90–1.16)
				1,968							
	IVS1 + 539		Control	(0.79)	520 (0.21)	788 (0.63)	392 (0.32)	64 (0.05)			
17	IPF1_SNP7			G	A	G/G	G/A	A/A			
				2,836		1,260					
	none	none	Case	(0.89)	344 (0.11)	(0.79)	316 (0.20)	14 (0.01)	0.1309	0.2318	1.14 (0.97–1.34)
				2,186							
	IVS1 + 1787		Control	(0.88)	302 (0.12)	953 (0.77)	280 (0.23)	11 (0.01)			

Continued on following page

TABLE 1
Continued

Number	Locus	HapMap data	Subject	Allele data (frequency) 1	Allele data (frequency) 3	Genotype data (frequency) 1	Genotype data (frequency) 2	Genotype data (frequency) 3	*P* value Allele 2*2	*P* value Genotype 2*3	OR (95% CI)
18	NEUROG3_SNP1			A	G	A/A	A/G	G/G			
	rs3812704	JPT	Case	1,472 (0.46)	1,708 (0.54)	337 (0.21)	798 (0.50)	455 (0.29)	0.2674	0.4687	1.06 (0.96–1.18)
	−1822		Control	1,114 (0.45)	1,374 (0.55)	252 (0.20)	610 (0.49)	382 (0.31)			
19	NEUROG3_SNP2			T	C	T/T	T/C	C/C			1.06 (0.95–1.19)
	rs4536103	JPT	Case	2,268 (0.71)	912 (0.29)	798 (0.50)	672 (0.42)	120 (0.08)	0.3129	0.2040	
	F199S		Control	1,743 (0.70)	745 (0.30)	616 (0.50)	511 (0.41)	117 (0.09)			
20	PAX6_SNP1			A	T	A/A	A/T	T/T			1.08 (0.97–1.20)
	rs2239789	none	Case	1,725 (0.54)	1,455 (0.46)	483 (0.30)	759 (0.48)	348 (0.22)	0.1697	0.2391	
	IVS6 + 282		Control	1,396 (0.56)	1,092 (0.44)	392 (0.32)	612 (0.49)	240 (0.19)			
21	PAX6_SNP2			C	T	C/C	C/T	T/T			1.01 (0.86–1.18)
	rs667773	none	Case	2,791 (0.88)	389 (0.12)	1,228 (0.77)	335 (0.21)	27 (0.02)	0.9358	0.8923	
	IVS7 + 218		Control	2,181 (0.88)	307 (0.12)	961 (0.77)	259 (0.21)	24 (0.02)			
22	NKX2-2_SNP1			T	C	T/T	T/C	C/C			1.08 (0.74–1.56)
	none	none	Case	3,117 (0.98)	63 (0.02)	1,530 (0.96)	57 (0.04)	3 (0.002)	0.7650	0.8727	
	+856 TGA		Control	2,435 (0.98)	53 (0.02)	1,193 (0.96)	49 (0.04)	2 (0.002)			
23	NKX2-2_SNP2			C	T	C/C	C/T	T/T			1.13 (1.02–1.25)
	rs3746741	none	Case	1,666 (0.52)	1,514 (0.48)	452 (0.28)	762 (0.48)	376 (0.24)	0.0251*	0.0563	
	+1163 TGA		Control	1,228 (0.49)	1,260 (0.51)	305 (0.25)	618 (0.50)	321 (0.26)			
24	NKX6-1_SNP1			A	C	A/A	A/C	C/C			1.03 (0.92–1.16)
	rs1017560	JPT	Case	2,182 (0.69)	998 (0.31)	747 (0.47)	688 (0.43)	155 (0.10)	0.6052	0.0144*	
	−15606		Control	1,724 (0.69)	764 (0.31)	625 (0.50)	474 (0.38)	145 (0.12)			
25	NKX6-1_SNP2			T	G	T/T	T/G	G/G			1.01 (0.83–1.23)
	none	none	Case	2,939 (0.92)	241 (0.08)	1,359 (0.85)	221 (0.14)	10 (0.01)	0.9750	0.9966	
	−8823		Control	2,298 (0.92)	190 (0.08)	1,062 (0.85)	174 (0.14)	8 (0.01)			