

Association of *CDKAL1*, *IGF2BP2*, *CDKN2A/B*, *HHEX*, *SLC30A8* and *KCNJ11* with susceptibility to type 2 diabetes in a Japanese population

Shintaro Omori M.D.^{1,2}, Yasushi Tanaka M.D., Ph.D.², Atsushi Takahashi Ph.D.³, Hiroshi Hirose M.D., Ph.D.⁴, Atsunori Kashiwagi M.D., Ph.D.⁵, Kohei Kaku M.D., Ph.D.⁶, Ryuzo Kawamori M.D., Ph.D.⁷, Yusuke Nakamura M.D., Ph.D.⁸,
Shiro Maeda M.D., Ph.D.¹

¹Laboratory for Diabetic Nephropathy, SNP Research Center, RIKEN,
Yokohama, Kanagawa, Japan

²Department of Internal Medicine, Division of Metabolism and Endocrinology,
St. Marianna University School of Medicine, Kawasaki, Kanagawa, Japan

³Laboratory for statistical analysis, SNP Research Center, RIKEN, Tokyo, Japan

⁴Health Center, Keio University School of Medicine, Tokyo, Japan

⁵Department of Medicine, Shiga University of Medical Science, Otsu, Shiga, Japan

⁶Division of Endocrinology and Metabolism, Department of Internal Medicine, Kawasaki
Medical School, Kurashiki, Okayama, Japan

⁷Department of Medicine, Metabolism and Endocrinology, School of Medicine, Juntendo
University, Tokyo, Japan

⁸Laboratory of Molecular Medicine, Human Genome Center,
Institute of Medical Science, University of Tokyo, Tokyo, Japan

Running title: Genes for type 2 diabetes in the Japanese

Correspondence:

Shiro Maeda

Laboratory for Diabetic Nephropathy, SNP Research Center, RIKEN
1-7-22 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045, Japan
smaeda@src.riken.jp

Received for publication 14 July 2007 and accepted in revised form 12 December 2007.

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

ABSTRACT

Objective: Recently, several genes have been shown to be associated with an increased risk of type 2 diabetes by genome-wide association studies in white populations. To further investigate the involvement of these polymorphisms in conferring susceptibility to type 2 diabetes, we examined the association of 14 SNPs within 11 candidate loci with type 2 diabetes in a Japanese population.

Research design and methods: We analyzed 14 SNPs (rs4402960 in *IGF2BP2*, rs10811661 in *CDKN2A/B*, rs1111875 and rs7923837 in *HHEX*, rs13266634 in *SLC30A8*, rs1113132 and rs11037909 in *EXT2*, rs9939609 and rs8050136 in *FTO*, rs7756992 in *CDKAL1*, rs1801282 in *PPARG* Pro12Ara, rs5219 in *KCNJ11* Glu23Lys, rs7480010 in *LOC387761*, and rs9300039 in Ch11) in 1,630 Japanese subjects with type 2 diabetes and in 1,064 control subjects by using an invader assay or a TaqMan assay.

Results: Among the 11 loci examined, 6 loci were significantly associated with type 2 diabetes in our population by a logistic regression analysis, similar to previously reported results (rs4402960, $p = 0.00009$; rs10811661, $p = 0.0024$; rs5219, $p = 0.0034$; rs1111875, $p = 0.0064$; rs13266634, $p = 0.0073$; rs7756992, $p = 0.0363$). In this population, the remaining 5 loci were not significantly associated with type 2 diabetes. In addition we identified significant association of the SNPs in *FTO* gene with BMI in the control subjects.

Conclusion: We have identified 6 of the 11 loci that were identified by genome-wide association studies in white populations, and these loci are considered strong candidates for type 2 diabetes susceptibility across different ethnicities.

Type 2 diabetes affects more than 200 million individuals worldwide, and its prevalence continues to increase in many countries, including Japan. Although the precise mechanisms underlying the development and progression of type 2 diabetes have not been elucidated, a combination of multiple genetic and/or environmental factors is considered to contribute to the pathogenesis of the disease (1).

Recently, genome-wide association studies conducted by several independent European and American groups have identified multiple susceptible variants in white populations including *TCF7L2* variants, those had been originally identified by a genome-wide linkage study (2), and confirmed in several replication studies across different ethnicities (3-7). Sladek et al. additionally identified the solute carrier family 30 member 8 (*SLC30A8*), homeobox hematopoietically expressed (*HHEX*), *LOC387761*, and exostosin 2 (*EXT2*) genes (8), and the WTCCC/UKT2D, FUSION, DGI study groups identified the insulin - like growth factor 2 mRNA binding protein 2 (*IGF2BP2*), cyclin dependent kinase 5 regulatory subunit associated protein 1 - like 1 (*CDKAL1*), cyclin-dependent kinase inhibitor - 2A/B (*CDKN2A/B*), fat mass and obesity associated (*FTO*) genes and the rs9300039 locus as additional loci that were strongly associated with the susceptibility to this disease (9-11). The latter study also confirmed the association between the susceptibility to type 2 diabetes and the peroxisome proliferator activated receptor gamma (*PPARG*) Pro12Ala or

potassium inwardly - rectifying channel subfamily J member 11 (*KCNJ11*) Glu23Lys polymorphism that had already been reported as strong candidates. The associations among single nucleotide polymorphisms (SNPs) within *CDKAL1* and *SLC30A8* were also identified by a genome-wide association study conducted for the Icelandic population (12).

These additional loci are also considered to be strong candidates for conferring susceptibility to type 2 diabetes in white populations. However, the contributions of these new loci should be evaluated in other ethnic populations, because it is well known that there are significant differences in the frequencies of some genetic variations among different ethnic groups (6, 7, 13).

The aim of the present study is to determine whether or not the variations identified by the genome-wide association studies in white populations are associated with the susceptibility to type 2 diabetes in a Japanese population.

RESEARCH DESIGN AND METHODS

Subject and DNA preparation. DNA samples were obtained from the peripheral blood samples of 1,630 type 2 diabetes patients recruited from the outpatient clinic of the Shiga University of Medical Science, Kawasaki Medical School (978 men and 652 women; age, 61.5 ± 11.6 years; duration of diabetes, 11.5 ± 13.9 years; HbA1c, $7.4 \pm 1.6\%$; fasting plasma glucose, 9.1 ± 3.5 mmol/l; BMI, 23.7 ± 3.9 kg/m² [all values are expressed as mean \pm SD], Table 1). Diabetes was diagnosed

according to the WHO criteria. Type 2 diabetes is clinically defined as a disease with gradual adult onset. Subjects who tested positive for anti-glutamic acid decarboxylase (GAD) antibodies and those diagnosed as mitochondrial disease (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes [MELAS]) or maturity onset diabetes of young (MODY) were not included in the case group. We also examined 1,064 control subjects who were enrolled from an annual health check conducted either at the Juntendo University or Keio University (Tokyo, Japan; 638 men and 426 women; age, 45.5 ± 9.5 years; HbA1c, $4.7 \pm 0.4\%$; fasting plasma glucose, 5.1 ± 0.5 mmol/l; BMI 22.9 ± 3.0 kg/m² [all values are expressed as means \pm SD], Table 1).

Written informed consents were obtained from all the participants, and DNA was extracted using the standard phenol-chloroform procedure. The protocol was approved by the ethics committee of the Institute of Physical and Chemical Research (RIKEN).

Genotyping. Each SNP genotyping was performed by the TaqMan assay (Applied Biosystems, Foster City, CA, U.S.A.) or by the multiplex-PCR invader assay as described previously (13). The success rates of these assays were >95%, and there was almost a 100% agreement between the results of genotyping and direct sequencing.

Statistical analysis. Statistical methods for determining associations, and to calculate linkage disequilibrium (LD) coefficients (r^2) were described previously (14). We performed Hardy-Weinberg Equilibrium (HWE) test according to the method described by

Nielsen DM et al (15). Although the genotype distributions of all the SNPs were within the Hardy-Weinberg equilibrium ($p \geq 0.01$), some of them had borderline results for HWE test (rs5219 in control, rs7480010 in case, rs8050136 in case, supplementary table 1 [available at <http://diabetes.diabetesjournals.org>]). Therefore, we performed Wright's F statistics (16) to evaluate the difference in the population structure between our case and control groups using randomly selected 96 SNPs. The result indicated that the population structures of our case and control groups were almost the same in view of a very small F_{ST} value between the both two groups ($F_{ST} = 0.001556$).

The differences between the case and control groups in terms of genotype distribution were analyzed using a logistic regression analysis. To test the additive model of each SNP after adjusting sex, age and log transformed BMI, the analysis was performed using StatView software. In addition, χ^2 test to evaluate the additive, dominant, and recessive models of each SNP were also performed by the method of Sladek et al (8).

The difference in the BMI according to the genotypes was analyzed using a multiple linear regression with log transformed BMI as the dependent variable and genotype as the independent variable with sex as a covariate for log transformed BMI (17).

RESULTS

As shown in Table 2, 6 SNPs within 6 distinct loci (rs4402960, rs10811661, rs5219, rs1111875, rs13266634, rs7756992) were found to be

significantly associated with type 2 diabetes in our Japanese population. No significant association was observed between the remaining 5 loci and type 2 diabetes in this population ($p \geq 0.05$; Table 2). We also evaluated the association of the 14 SNP loci with type 2 diabetes using χ^2 test by the method of Sladek et al (8) (supplementary Table 2), and identified almost consistent results with those obtained by a logistic regression analysis even after selecting control subjects whose age over 50 years old ($n=382$, supplementary Table 3). Since *FTO* variants have been reported to be associated with BMI, we also examined the association of these polymorphisms with BMI in our control subjects, and we found that the polymorphisms within *FTO* and *HHEX* were modestly associated with BMI (Table 3).

DISCUSSION

In the present study, we identified significant associations of SNPs within the *CDKAL1*, *IGF2BP2*, *CDKN2A/B*, *HHEX*, *SLC30A8* and *KCNJ11* genes with the susceptibility to type 2 diabetes in a Japanese population. We also found that the SNPs in the *FTO* gene, and *HHEX* gene were significantly associated with BMI in our control group.

Recent advances in human genetic research have facilitated the identification of genes conferring susceptibility to common diseases such as type 2 diabetes from across the entire human genome by using a large number of subjects, and genome-wide association studies have been conducted worldwide (8–12). Although the importance of *TCF7L2* as a

susceptibility gene for type 2 diabetes has been well established, its polymorphism could account for approximately 20% of all cases for type 2 diabetes in white populations (2, 4, 5). The population-attributable risk of the *TCF7L2* polymorphism in the Japanese was approximately 2% because the risk allelic frequency in a previously studied Japanese population was very low (6,7). Further, many important genes for the disease remain to be identified, especially in East Asian populations.

Several groups have independently performed genome-wide association studies for type 2 diabetes in white populations (8–12). All these studies have demonstrated that the *TCF7L2* polymorphism is most strongly associated with the susceptibility to type 2 diabetes, and from a large set of replication studies, they have identified additional candidate loci also associated with the disease. The results of these genome-wide association studies are also considered highly consistent with regard to white populations. However, there are considerable differences in phenotype between Japanese (lean and less hyperinsulinemic Asian type 2 diabetes) and White (European descent) type 2 diabetes, and these differences might affect the genetic contribution of each gene to conferring susceptibility to type 2 diabetes. Therefore, the new candidates should also be evaluated in different ethnic groups because there are clear ethnic differences in terms of genetic contribution to diseases (13, 18).

In the present study, we also identified the 6 SNPs within the *CDKAL1*, *IGF2BP2*, *CDKN2A/B*, *HHEX*, *SLC30A8* and *KCNJ11* genes that were significantly associated with

type 2 diabetes in our Japanese population. Since the risk alleles for these variations in the tested population were entirely consistent with those in white populations (8–12), the contribution of these polymorphisms to type 2 diabetes susceptibility is highly convincing across the different ethnicities, although there are considerable differences in allele frequencies between the Japanese and white populations (Table 4 and supplementary table 4). Regarding the *HHEX* locus, only rs1111875 had significant association with type 2 diabetes in the present study, whereas both rs1111875 and rs7923837 were associated with the disease in white populations. The risk allele frequencies of SNPs in *HHEX* gene were significantly different between our population and white populations (rs1111875: 28.4% vs. 56.1%, rs7923837: 20.6% vs. 60.1% Japanese and white population, respectively, supplementary Table 4), and there were some differences in the LD coefficients (r^2) between rs1111875 and rs7923837 among those populations (0.5 in Japanese, 0.698 in white population). Therefore, these differences might explain the discrepancies in the results for the association of SNPs in *HHEX* gene with type 2 diabetes, although the possibility of insufficient study power in the present study could not be excluded.

We also found that the SNPs within *FTO* (rs9939609 and rs8050136) and *HHEX* (rs1111875 and rs7923837) were modestly associated with BMI in our control subjects (Table 3). The association of the SNPs in *FTO* with type 2 diabetes was not significant in

our population (Table 2, supplementary Table 2, 3). Therefore, the variations in the *FTO* gene might directly affect body weight rather than type 2 diabetes itself; this finding is also consistent with that in white populations (17, 19).

Among the other 4 loci, the association of the Pro12Ala polymorphism in the *PPARG* gene with type 2 diabetes has been well established (20, 21), and this association was also observed in Japanese populations (22, 23), although we could not determine whether the association of this polymorphism with type 2 diabetes was significant. Because the frequencies of the Ala allele or its carrier (X/Ala) found in the present study (3% and 6%, respectively) are consistent with those in previous studies on Japanese populations, the frequencies of the Ala allele can be considered very low in the Japanese as compared to those in white populations. Since an estimated power to detect the association of the SNP with type 2 diabetes in the present study is approximately 20%, the results of the present study do not appear strong enough to determine the association between the *PPARG* Pro12Ala polymorphism and type 2 diabetes.

With regard to the remaining 3 loci, the results for *EXT2*, *LOC387761*, and rs930039 were not always in agreement with those of the genome-wide association studies in white populations. In addition, the allele frequencies of those SNPs were also significantly different between the Japanese and the white populations (Table 4 and supplementary Table 4). Since the estimated powers of the present study were >90%, >90%, >70%

for rs1113132 (*EXT2*), rs7480010 (*LOC387761*), rs930039, respectively, the contribution of these 3 loci in the Japanese populations is considered minor, if present at all; however more replication studies are required for the precise evaluation of these loci.

In conclusion, we identified significant associations between SNPs within the *CDKAL1*, *IGF2BP2*, *CDKN2A/B*, *HHEX*, *SLC30A8* and *KCNJ11* genes and type 2 diabetes in a Japanese population. These loci are considered strong candidates for conferring susceptibility to type 2 diabetes across different ethnicities.

However, further studies are required to elucidate the association of other loci with the susceptibility to type 2 diabetes and the biological significance of these genes and gene polymorphisms.

ACKNOWLEDGEMENTS

We thank Shuichi Tsukada, Masaki Kobayashi, and the technical staff of the Laboratory for Diabetic Nephropathy at the SNP Research Center for providing technical assistance. This work was partly supported by the Japanese Millennium Project.

REFERENCES

1. O’Rahilly S, Barroso I, Wareham NJ: Genetic factors in type 2 diabetes: the end of the beginning? *Science* 307: 370-373, 2005
2. Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, Helgason A, Stefansson H, Emilsson V, Helgadottir A, Styrkarsdottir U, Magnusson KP, Walters GB, Palsdottir E, Jonsdottir T, Gudmundsdottir T, Gylfason A, Saemundsdottir J, Wilensky RL, Reilly MP, Rader DJ, Bagger Y, Christiansen C, Gudnason V, Sigurdsson G, Thorsteinsdottir U, Gulcher JR, Kong A, Stefansson K: Variant of transcription factor 7-like 2 (*TCF7L2*) gene confers risk of type 2 diabetes. *Nat Genet* 38:320-323, 2006
3. Florez JC, Jablonski KA, Bayley N, Pollin TI, de Bakker PI, Shuldiner AR, Knowler WC, Nathan DM, Altshuler D; Diabetes Prevention Program Research Group: *TCF7L2* polymorphisms and progression to diabetes in the diabetes prevention program. *N Engl J Med* 355:241-250, 2006
4. Groves CJ, Zeggini E, Minton J, Frayling TM, Weedon MN, Rayner NW, Hitman GA, Walker M, Wiltshire S, Hattersley AT, McCarthy MI: Association Analysis of 6,736 UK subjects provides replication and confirms *TCF7L2* as a type 2 diabetes susceptibility gene with a substantial effect on individual risk. *Diabetes* 55:2640-2644, 2006
5. Zhang C, Qi L, Hunter DJ, Meigs JB, Manson JE, van Dam RM, Hu FB: 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, 2006
6. Hayashi T, Iwamoto Y, Kaku K, Hirose H, Maeda S: Replication study for the association of *TCF7L2* with susceptibility to the type 2 diabetes in a Japanese population. *Diabetologia* 50:980-984, 2007
7. Horikoshi M, Hara K, Ito C, Nagai R, Froguel P, Kadowaki T: 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, 2007
8. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, Boutin P, Vincent D, Belisle A, Hadjadj S, Balkau B, Heude B, Charpentier G, Hundson TJ, Montpetit A, Pshezhetsky AV, Prentki M, Posner BI, Balding DJ, Meyre D, Polychronakos C, Froguel P: A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 445:881-885, 2007
9. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, Timpson NJ, Perry JR, Rayner NW, Freathy RM, Barrett JC, Shields B, Morris AP, Ellard S, Groves CJ, Harries LW, Marchini JL, Owen KR, Knight B, Cardon LR, Walker M, Hitman GA, Morris AD, Doney AS; Wellcome Trust Case Control Consortium (WTCCC), Burton PR, Clayton DG, Craddock N, Deloukas P, Duncanson A, Kwiatkowski DP, Ouwehand WH, Samani NJ, Todd JA, Donnelly P, Davison D, Easton D, Evans D, Leung HT, Spencer CC, Tobin MD, Attwood AP, Boorman JP, Cant B, Everson U, Hussey JM, Jolley JD, Knight AS, Koch K, Meech E, Nutland S, Prowse CV, Stevens HE, Taylor NC, Walters GR, Walker NM, Watkins NA, Winzer T, Jones RW, McArdle WL, Ring SM, Strachan DP, Pembrey M, Breen G, St Clair D, Caesar S, Gordon-Smith K, Jones L, Fraser C, Green EK,

Grozeva D, Hamshere ML, Holmans PA, Jones IR, Kirov G, Moskvina V, Nikolov I, O'donovan MC, Owen MJ, Collier DA, Elkin A, Farmer A, Williamson R, McGuffin P, Young AH, Ferrier IN, Ball SG, Balmforth AJ, Barrett JH, Bishop DT, Iles MM, Maqbool A, Yuldasheva N, Hall AS, Braund PS, Dixon RJ, Mangino M, Stevens S, Thompson JR, Bredin F, Tremelling M, Parkes M, Drummond H, Lees CW, Nimmo ER, Satsangi J, Fisher SA, Forbes A, Lewis CM, Onnie CM, Prescott NJ, Sanderson J, Mathew CG, Barbour J, Mohiuddin MK, Todhunter CE, Mansfield JC, Ahmad T, Cummings FR, Jewell DP, Webster J, Brown MJ, Lathrop GM, Connell J, Dominiczak A, Braga Marcano CA, Burke B, Dobson R, Gungadoo J, Lee KL, Munroe PB, Newhouse SJ, Onipinla A, Wallace C, Xue M, Caulfield M, Farrall M, Barton A, Bruce IN, Donovan H, Eyre S, Gilbert PD, Hider SL, Hinks AM, John SL, Potter C, Silman AJ, Symmons DP, Thomson W, Worthington J, Dunger DB, Widmer B, Newport M, Sirugo G, Lyons E, Vannberg F, Hill AV, Bradbury LA, Farrar C, Pointon JJ, Wordsworth P, Brown MA, Franklyn JA, Heward JM, Simmonds MJ, Gough SC, Seal S, Stratton MR, Rahman N, Ban M, Goris A, Sawcer SJ, Compston A, Conway D, Jallow M, Rockett KA, Bumpstead SJ, Chaney A, Downes K, Ghori MJ, Gwilliam R, Hunt SE, Inouye M, Keniry A, King E, McGinnis R, Potter S, Ravindrarajah R, Whittaker P, Widdén C, Withers D, Cardin NJ, Ferreira T, Pereira-Gale J, Hallgrimsdottir IB, Howie BN, Su Z, Teo YY, Vukcevic D, Bentley D, Compston A, Ouwehand NJ, Samani MR, Isaacs JD, Morgan AW, Wilson GD, Ardern-Jones A, Berg J, Brady A, Bradshaw N, Brewer C, Brice G, Bullman B, Campbell J, Castle B, Cetnarsryj R, Chapman C, Chu C, Coates N, Cole T, Davidson R, Donaldson A, Dorkins H, Douglas F, Eccles D, Eeles R, Elmslie F, Evans DG, Goff S, Goodman S, Goudie D, Gray J, Greenhalgh L, Gregory H, Hodgson SV, Homfray T, Houlston RS, Izatt L, Jackson L, Jeffers L, Johnson-Roffey V, Kavalier F, Kirk C, Laloo F, Langman C, Locke I, Longmuir M, Mackay J, Magee A, Mansour S, Miedzybrodzka Z, Miller J, Morrison P, Murday V, Paterson J, Pichert G, Porteous M, Rahman N, Rogers M, Rowe S, Shanley S, Sagar A, Scott G, Side L, Snadden L, Steel M, Thomas M, Thomas S, McCarthy MI, Hattersley AT: Replication of genome-wide association signal in U.K. samples reveals risk loci for type 2 diabetes. *Science* 316:1336-1341, 2007

10. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding CJ, Swift AJ, Narisu N, Hu T, Pruim R, Xiao R, Li XY, Conneely KN, Riebow NL, Sprau AG, Tong M, White PP, Hetrick KN, Barnhart MW, Bark CW, Goldstein JL, Watkins L, Xiang F, Saramies J, Buchanan TA, Watanabe RM, Valle TT, Kinnunen L, Abecasis GR, Pugh EW, Doheny KF, Bergman RN, Tuomilehto J, Collins FS, Boehnke M: A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 316:1341-1345, 2007

11. Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research, Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PI, Chen H, Roix JJ, Kathiresan S, Hirschhorn JN, Daly MJ, Hughes TE, Groop L, Altshuler D, Almgren P, Florez JC, Meyer J, Ardlie K, Bengtsson Bostrom K, Isomaa B, Lettre G, Lindblad U, Lyon HN, Melander O, Newton-Cheh C, Nilsson P, Orho-Melander M, Rastam L, Speliotes

- EK, Taskinen MR, Tuomi T, Guiducci C, Berglund A, Carlson J, Gianniny L, Hackett R, Hall L, Holmkvist J, Laurila E, Sjogren M, Sterner M, Surti A, Svensson M, Svensson M, Tewhey R, Blumenstiel B, Parkin M, Defelice M, Barry R, Brodeur W, Camarata J, Chia N, Fava M, Gibbons J, Handsaker B, Healy C, Nguyen K, Gates C, Sougnez C, Gage D, Nizzari M, Gabriel SB, Chirn GW, Ma Q, Parikh H, Richardson D, Rieke D, Purcell S: Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 316:1331-1336, 2007
12. Steinthorsdottir V, Thorleifsson G, Reynisdottir I, Benediktsson R, Jonsdottir T, Walters GB, Styrkarsdottir U, Gretarsdottir S, Emilsson V, Ghosh S, Baker A, Snorraddottir S, Bjarnason H, Ng MC, Hansen T, Bagger Y, Wilensky RL, Reilly MP, Adeyemo A, Chen Y, Zhou J, Gudnason V, Chen G, Huang H, Lashley K, Doumatey A, So WY, Ma RC, Andersen G, Borch-Johnsen K, Jorgensen T, van Vliet-Ostaptchouk JV, Hofker MH, Wijmenga C, Christiansen C, Rader DJ, Rotimi C, Gurney M, Chan JC, Pedersen O, Sigurdsson G, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K: A variant in *CDKAL1* influences insulin response and risk of type 2 diabetes. *Nat Genet* 39:770-775, 2007
13. S Maeda, S Tsukada, A Kanazawa, A Sekine, T Tsunoda, D Koya, H Maegawa, A Kashiwagi, T Babazono, M Matsuda, Y Tanaka, T Fujioka, H Hirose, T Eguchi, Y Ohno, Christopher JG, Andrew TH, Graham AH, Mark W, K Kaku, Y Iwamoto, R Kawamori, R Kikkawa, N Kamatani, Mark I McCarthy, Y Nakamura: Genetic variations in the gene encoding *TFAP2B* are associated with type 2 diabetes mellitus. *J Hum Genet* 50:283-292, 2005
14. Devlin B, Risch N: A comparison of linkage disequilibrium measures for fine-scale mapping. *Genomics* 20:311-322, 1995
15. Nielsen DM, Ehm MG, Weir BS: Detecting marker-disease association by testing for Hardy-Weinberg disequilibrium at a marker locus. *Am J Hum Genet* 63:1531-1540, 1998
16. Wright S: Evolution and the Genetics of Populations volume 2: The theory of gene frequencies. *Univ. of Chicago Press*: 295-295, 1969
17. Frayling TM, Timpson NJ, Weedon MN, Zeggini E, Freathy RM, Lindgren CM, Perry JR, Elliott KS, Lango H, Rayner NW, Shields B, Harries LW, Barrett JC, Ellard S, Groves CJ, Knight B, Patch AM, Ness AR, Ebrahim S, Lawlor DA, Ring SM, Ben-Shlomo Y, Jarvelin MR, Sovio U, Bennett AJ, Melzer D, Ferrucci L, Loos RJ, Barroso I, Wareham NJ, Karpe F, Owen KR, Cardon LR, Walker M, Hitman GA, Palmer CN, Doney AS, Morris AD, Smith GD, Hattersley AT, McCarthy MI: A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult Obesity. *Science* 316:889-894, 2007
18. The International HapMap Consortium: A haplotype map of the human genome. *Nature* 437:1299-1320, 2005
19. Dina C, Meyre D, Gallina S, Durand E, Korner A, Jacobson P, Carlsson LM, Kiess W, Vatin V, Lecoeur C, Delplanque J, Vaillant E, Pattou F, Ruiz J, Weill J, Levy-Marchal C, Horber F, Potoczna N, Hercberg S, Le Stunff C, Bougneres P, Kovacs P, Marre M, Balkau B, Cauchi S, Chevre JC, Froguel P: Variation in *FTO* contributes to childhood obesity and severe adult obesity. *Nat Genet* 39:724-726, 2007

20. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES: The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. *Nat Genet* 26:76-80, 2000
21. Tonjes A, Loeffler M, Scholz M, Stumvoll M: Association of Pro12Ala Polymorphism in peroxisome proliferator-activated receptor gamma with pre-diabetic phenotypes. *Diabetes Care* 29:2489-2497, 2006
22. Hara K, Okada T, Tobe K, Yasuda K, Mori Y, Kadowaki H, Hagura R, Akanuma Y, Kimura S, Ito C, Kadowaki T: The Pro12Ala polymorphism in PPAR gamma 2 may confer resistance to type 2 diabetes. *Biochem Biophys Res Commun* 271:212-216, 2000
23. Kawasaki I, Tahara H, Emoto M, Shoji T, Shioji A, Okuno Y, Inaba M, Nishizawa Y: Impact of Pro12Ala variant in the peroxisome proliferators-activated receptor (PPAR) gamma2 on obesity and insulin resistance in Japanese type 2 diabetic and healthy subjects. *Osaka City Med J* 48:23-28, 2002

TABLE 1. Clinical characteristics of the subjects

	Type 2 diabetes n = 1,630	Control n = 1,064	p value*
Sex (M : F)	978 : 652	638 : 426	0.9845
Age (years)#	61.5 ± 11.6	45.5 ± 9.5	<0.0001
BMI (kg/m ²)#	23.7 ± 3.9	22.9 ± 3.0	<0.0001
FPG (mmol/l)#	9.1 ± 3.5	5.1 ± 0.5	<0.0001
HbA1c (%)#	7.4 ± 1.6	4.7 ± 0.4	<0.0001
Duration of diabetes (years)#	11.5 ± 13.9	-	-

* Pearson's chi-square test # Values are expressed as mean ± SD

TABLE 2. Association of candidate SNP loci with type 2 diabetes

SNP	gene	p value [#]	odds ratio (95% C.I)
rs4402960	<i>IGF2BP2</i>	0.00009	1.368 (1.169 - 1.600)
rs10811661	<i>CDKN2A/B</i>	0.0024	1.255 (1.084 - 1.454)
rs5219	<i>KCNJ11</i>	0.0034	1.254 (1.078 - 1.459)
rs1111875#	<i>HHEX</i>	0.0064	1.243(1.063 - 1.453)
rs7923837#	<i>HHEX</i>	0.3773	1.083 (0.907 - 1.293)
rs13266634	<i>SLC30A8</i>	0.0073	1.225 (1.056 - 1.420)
rs7756992	<i>CDKAL1</i>	0.0363	1.164 (1.010-1.342)
rs9939609	<i>FTO</i>	0.2376	1.114 (0.931 - 1.332)
rs8050136	<i>FTO</i>	0.3520	1.089 (0.910-1.302)
rs1801282	<i>PPARG</i>	0.4137	0.843 (0.559 - 1.270)
rs7480010	<i>LOC387761</i>	0.4393	1.073 (0.898 - 1.281)
rs1113132	<i>EXT2</i>	0.4728	1.056 (0.910 - 1.225)
rs11037909	<i>EXT2</i>	0.5365	1.048 (0.903 - 1.216)
rs9300039	41871942§	0.6966	1.034 (0.874 - 1.222)

* P value is calculated on logistic regression with additive model (Sex, Age, BMI adjusted, BMI are Log-transformed for the analysis.). # $r^2 = 0.50$ (this study), 0.346 (HapMap-JPT), 0.698 (HapMap-CEU), respectively. § Position on the chromosome is indicated.

TABLE 3. Association of the SNP loci with BMI in control subjects

SNP / gene	genotype (number of subjects) / BMI*			p value
rs9939609	TT (676)	AT (331)	AA (37)	
<i>FTO</i>	22.2 (21.9, 22.4)	23.0 (22.7, 23.4)	23.1 (22.5, 23.8)	0.0271
rs8050136	CC (678)	CA (331)	AA (35)	
<i>FTO</i>	22.2 (22.0, 22.4)	23.0 (22.7, 23.3)	23.2 (22.5, 23.9)	0.0436
rs7923837	AA (653)	AG (333)	GG (46)	
<i>HHEX</i>	22.4 (22.2, 22.6)	22.5 (22.2, 22.8)	22.7 (21.9, 23.5)	0.032
rs1111875	TT (529)	CT (419)	CC (84)	
<i>HHEX</i>	22.48 (22.2, 22.7)	22.5 (22.2, 22.7)	22.3 (21.7, 22.9)	0.05
rs5219	CC (421)	CT (509)	TT (118)	
<i>KCNJ11</i>	22.4 (22.1, 22.7)	22.6 (22.3, 22.8)	22.1 (21.6, 22.6)	0.0777
Glu23Lys				
rs1801282	CC (2)	CG (53)	GG (995)	
<i>PPARG</i>	24.4	23.13 (22.4, 23.9)	22.4 (22.2, 22.6)	0.1039
pro12ala				
rs11037909	CC (141)	CT (471)	TT (425)	
<i>EXT2</i>	22.4 (21.9, 22.9)	22.7 (22.4, 22.9)	22.3 (22.0, 22.6)	0.1243
rs1113132	CC (143)	CG (467)	GG (427)	
<i>EXT2</i>	22.4 (21.9, 22.9)	22.6 (22.4, 22.9)	22.3 (22.0, 22.6)	0.1315
rs9300039	AA (68)	AC (370)	CC (592)	
41871942#	22.7 (21.9, 23.5)	22.5 (22.2, 22.8)	22.4 (22.2, 22.6)	0.4153
rs13266634	TT (173)	CT (491)	CC (376)	
<i>SLC30A8</i>	22.6 (22.1, 23.1)	22.5 (22.2, 22.7)	22.4 (22.2, 22.7)	0.6014

rs10811661	CC (200)	CT (518)	TT (326)	
<i>CDKN2A/B</i>	22.3 (21.9, 22.7)	22.5 (22.2, 22.7)	22.5 (22.2, 22.8)	0.6185
rs7480010	AA (684)	AG (317)	GG (42)	
<i>LOC387761</i>	22.4 (22.1, 22.6)	22.8 (22.5, 23.1)	22.3 (21.5, 23.2)	0.7183
rs7756992	AA (289)	AG (508)	GG (236)	
<i>CDKAL1</i>	22.4 (22.0, 22.7)	22.6 (22.4, 22.9)	22.3 (22.0, 22.7)	0.7985
rs4402960	GG (520)	GT (433)	TT (88)	
<i>IGF2BP2</i>	22.5 (22.3, 22.8)	22.4 (22.1, 22.7)	22.5 (21.8, 23.1)	0.9313

*Data are presented as geometric means, and values for 95% C.I are in parenthesis. # Position on the chromosome is indicated.

TABLE 4. The comparison of risk allele frequency and population attributable risk between Japanese and white populations

SNP	gene	risk allele frequency		p value*	PAR (%)	
		(%)			This study	White populations #
		this study	White populations			
rs4402960	<i>IGF2BP2</i>	29.3	31.3	0.1439	11.1	4.8
rs10811661	<i>CDKN2A/B</i>	56.1	84.1	2.2×10^{-183}	13.4	18.1
rs5219	<i>KCNJ11</i>	35.5	46.4	1.9×10^{-16}	7.3	9.0
rs1111875	<i>HHEX</i>	28.4	57.7	3.2×10^{-146}	-	20.0
rs7923837	<i>HHEX</i>	20.6	62.3	3.6×10^{-218}	9.1	5.7
rs13266634	<i>SLC30A8</i>	60.0	63.4	0.0097	7.7	6.1
rs7756992	<i>CDKAL1</i>	47.4	23.2	3.0×10^{-114}	12.5	14.6
rs9939609	<i>FTO</i>	19.4	38.5	1.3×10^{-19}	-	-
rs8050136	<i>FTO</i>	19.3	39.0	6.0×10^{-67}	-	-
rs1801282	<i>PPARG</i>	97.3	82.4	4.2×10^{-63}	-	14.2
rs7480010	<i>LOC387761</i>	19.1	30.1	9.5×10^{-21}	-	30.6
rs1113132	<i>EXT2</i>	63.6	73.3	1.8×10^{-16}	-	8.0
rs11037909	<i>EXT2</i>	63.6	72.9	2.6×10^{-14}	-	19.0
rs9300039	41871942§	75.3	89.2	2.5×10^{-46}	-	25.0

* P values for χ^2 test for genotype distribution (2×3 contingency table). # Combined data from WTCCC / UKRS / FUSION /French / Icelandic studies.. §Position on the chromosome is indicated