Diabetologia (2011) 54:1350–1359
DOI 10.1007/s00125-011-2086-8

ARTICLE

# Association of genetic variants for susceptibility to obesity with type 2 diabetes in Japanese individuals

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Received: 5 September 2010 / Accepted: 25 January 2011 / Published online: 3 March 2011


**Abstract**
*Aims/hypothesis* In populations of East Asian descent, we performed a replication study of loci previously identified in populations of European descent as being associated with obesity measures such as BMI and type 2 diabetes.

*Methods* We genotyped 14 single nucleotide polymorphisms (SNPs) from 13 candidate loci that had previously been identified by genome-wide association meta-analyses for obesity measures in Europeans. Genotyping was done in 18,264 participants from two general Japanese populations. For SNPs showing an obesity association in Japanese individuals, we further examined diabetes associations in up to 6,781 cases and 7,307 controls from a subset of the original, as well as from additional populations.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00125-011-2086-8) contains supplementary material, which is available to authorised users.

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*Results* Significant obesity associations ($p<0.1$ two-tailed, concordant direction with previous reports) were replicated for 11 SNPs from the following ten loci in Japanese participants: *SEC16B*, *TMEM18*, *GNPDA2*, *BDNF*, *MTCH2*, *BCDIN3D–FAIM2*, *SH2B1–ATP2A1*, *FTO*, *MC4R* and *KCTD15*. The strongest effect was observed at *TMEM18* rs4854344 ($p=7.1\times10^{-7}$ for BMI). Among the 11 SNPs showing significant obesity association, six were also associated with diabetes (OR 1.05−1.17; $p=0.04-2.4\times10^{-7}$) after adjustment for BMI in the Japanese. When meta-analysed with data from the previous reports, the BMI-adjusted diabetes association was found to be highly significant for the *FTO* locus in East Asians (OR 1.13; 95% CI 1.09−1.18; $p=7.8\times10^{-10}$) with substantial inter-ethnic heterogeneity ($p=0.003$).

*Conclusions/interpretation* We confirmed that ten candidate loci are associated with obesity measures in the general Japanese populations. Six (of ten) loci exert diabetogenic effects in the Japanese, although relatively modest in size, and independently of increased adiposity.

**Keywords** Asians · Association study · Ethnicity · Obesity · Type 2 diabetes

**Abbreviations**

| | |
|---|---|
| CAGE | Cardiovascular Genome Epidemiology |
| GWA | Genome-wide association |
| SNP | Single nucleotide polymorphism |

## Introduction

Obesity is a major risk factor for type 2 diabetes, dyslipidaemia, hypertension and cardiovascular disease, and has a strong genetic component [1]. Twin studies have generally found heritability estimates of 0.75 to 0.85 for BMI and approximately 0.70 for weight [2]. Genome-wide association (GWA) studies have provided evidence that several loci are associated with common obesity mostly in populations of European descent [3–15]. The first such loci reported was the fat-mass and obesity-associated gene (*FTO*) [3, 16, 17]. A common variant in the *FTO* locus, rs9939609, was originally identified as part of a GWA study for type 2 diabetes [3]; people with homozygous risk alleles weighed approximately 3 kg more than those without a risk allele. It has been suggested that this variant can predispose individuals to type 2 diabetes and metabolic disorders through its primary effect on BMI in Europeans [18].

Ethnic differences have been assumed to exist between Europeans and Asians in terms of the components and impacts of genetic factors for obesity, and traits or disorders related to obesity (e.g. type 2 diabetes) [2, 19, 20]. For instance, the relationship between BMI and body fat per cent differs between these populations, with Asians in general having a higher body fat per cent at a lower BMI than Europeans [20]. It has also been estimated that the absolute genetic variances for BMI and weight are greater in Europeans than in East Asians, according to an adolescent twin study [2]. From an epidemiological viewpoint, moreover, it has been hypothesised that the overall impact of obesity on type 2 diabetes is greater in Asians than in Europeans [21]. Accordingly, it is of interest to compare the genetic associations between populations of European descent and Japanese populations.

To date, several studies on non-European populations have replicated the associations for a number of novel obesity loci previously identified by GWA studies in Europeans [13, 22–25]; nevertheless, statistical power has not been sufficient to make strong conclusions. Therefore to test associations between obesity measures (BMI and weight) and type 2 diabetes for 14 single nucleotide polymorphisms (SNPs) from 13 candidate loci recently reported by two GWA meta-analyses [10, 11], we performed a replication study in the general Japanese populations.

## Methods

### Study populations

We performed a replication study of previously identified variants in the general Japanese populations (Table 1, electronic supplementary material [ESM] Study samples for BMI association analysis). Specifically, 5,695 Japanese participants (referred to hereafter as the Amagasaki panel) were consecutively enrolled in the population-based setting as described previously [26] and 12,569 other Japanese

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**Table 1** Clinical characteristics of study participants for BMI

| Variables | Amagasaki panel | Fukuoka panel |
|---|---|---|
| Both sexes (*n*) | 5,695 | 12,569 |
| Women (*n*) | 2,290 | 6,898 |
| Men (*n*) | 3,405 | 5,671 |
| Age (years) | 48.8±12.6 | 62.6±6.8 |
| BMI (kg/m$^2$) | 23.0±3.2 | 23.1±3.0 |
| Body weight (kg) | 61.8±11.5 | 58.4±10.2 |
| Alcohol drinking (%) | | |
| None | 24.1 | 48.6 |
| Previous drinker | 1.2 | 5.0 |
| Chance drinker [a] | 35.6 | – |
| Current drinker | 39.1 | 46.4 |
| Smoking (%) | | |
| None | 55.2 | 59.9 |
| Previous smoker | 9.9 | 23.1 |
| Current smoker | 34.8 | 17.0 |
| Blood chemistry | | |
| Fasting plasma glucose (mmol/l) | 5.22±0.54 | N/A |
| $HbA_{1c}$ (%)[b] | 5.41±0.84 | 5.23±0.77 |
| LDL-cholesterol (mmol/l)[c] | 3.21±0.81 | N/A |
| Triacylglycerol (mmol/l) | 1.24±0.97 | 1.66±1.12 |
| HDL-cholesterol (mmol/l) | 1.63±0.46 | 1.62±0.44 |
| Blood pressure (mmHg) | | |
| Systolic blood pressure | 124.3±18.1 | 138.8±21.2 |
| Diastolic blood pressure | 75.9±11.6 | 83.9±11.7 |
| Prevalence of metabolic diseases (%)[d] | | |
| Hypertension | 23.4 | 56.9 |
| Diabetes | 6.1 | 7.6 |
| Dyslipidaemia | 42.2 | N/A |

Values are means±SD unless otherwise indicated

All clinical assessments were performed using uniform standards in each population; blood samples were taken after ≥6 h fast in the Amagasaki panel and without setting strict fasting condition in the Fukuoka panel

N/A, not applicable

[a] Since the questionnaire did not differentiate between chance drinker and current drinker, the corresponding participants were combined in the current drinker category in the Fukuoka panel

[b] $HbA_{1c}$ was measured in 1,288 participants in the Amagasaki panel and in all participants in the Fukuoka panel

[c] LDL-cholesterol was calculated in the Amagasaki panel using the Friedewald formula, with missing values assigned to individuals with triacylglycerol >4.52 mmol/l. Since blood samples were taken without setting strict fasting condition, the values for LDL-cholesterol and prevalence of dyslipidaemia are not shown for the Fukuoka panel, in accordance with the Japan Atherosclerosis Society Guidelines [49]

[d] Hypertension was defined as systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg, or taking antihypertensive medication. Diabetes was defined as fasting plasma glucose ≥7.0 mmol/l and/or $HbA_{1c}$≥6.5%, or taking blood glucose-lowering medication. Dyslipidaemia was defined according to the Japan Atherosclerosis Society Guidelines [49]. The criteria for diabetes are not identical to those adopted for selecting diabetic cases in the Fukuoka panel (see details in ESM Study samples for type 2 diabetes case–control studies)

participants (referred to hereafter as the Fukuoka panel) were randomly selected from residents aged 50 to 74 years in the general population [27]. Among the candidate loci tested in the two panels, those showing a replication of association ($p<0.05$ one-tailed, i.e. $p<0.1$ two-tailed, in concordant direction with previous reports) were subjected to tests to examine type 2 diabetes associations in a Japanese case–control study panel (ESM Table 1, ESM Study samples for type 2 diabetes case–control studies). For the purpose of uniformity, two tailed *p* values are shown throughout the text, unless otherwise indicated. Of 2,041 cases and 2,418 controls enrolled from the Cardiovascular Genome Epidemiology (CAGE) Network [28], 931 cases and 1,404 controls were included in stage 1 and 1,110 cases and 1,014 controls in stage 2. In addition, participants in the Fukuoka panel were included in stage 1 and Biobank Japan (http://biobankjp.org/ [in Japanese], accessed 1 February 2011) cases were included in stage 2 (ESM Table 1). From the CAGE Network, type 2 diabetes cases were enrolled according to the 1999 WHO criteria, while unaffected controls were enrolled according to the following criteria: (1) no past history of urinary glucose or glucose intolerance; (2) $HbA_{1c}<5.6\%$ or a normal result from 75 g glucose tolerance test; and (3) age at examination ≥55 years. From the population-based participants in the Fukuoka panel, diabetic participants ($n=740$) and unaffected controls ($n=4,889$) were selected for case–control analysis; among these samples, there was some overlap between the BMI/weight and type 2 diabetes studies. Here, diabetes was defined as $HbA_{1c}\geq7.0$ or under treatment for type 2 diabetes; a relatively stringent criterion for $HbA_{1c}$ (≥7.0%) was adopted because of the lack of fasting plasma glucose data for participants in the Fukuoka panel. The controls were chosen as non-diabetic participants who met the following conditions: age ≥55 years; $HbA_{1c}\leq5.0\%$; no previous and/or current treatment for diabetes; and absence of renal failure (serum creatinine<265.2 μmol/l), as previously described [27]. In total, 6,781 cases and 7,307 controls were used for the type 2 diabetes case–control study in Japanese. The CAGE Network samples were categorised, according to the stages of a previous GWA study [28], into two panels: CAGE–GWAS (stage 1); and CAGE–replication (stage 2) (ESM Table 1); genotyping in the CAGE-GWAS panel was performed with Infinium assay (Illumina, San Diego, CA). All participants enrolled in these different studies provided written informed consent. Local Ethics Committees approved the protocols used.

Height and body weight were measured by trained personnel using standard anthropometric techniques for all participants other than Biobank Japan type 2 diabetes participants, for whom the relevant data were self-reported from questionnaire.

### SNP genotyping and quality control

Samples (except for the CAGE–GWAS panel) were genotyped using the TaqMan assay (Applied Biosystems by Life Technologies, Carlsbad, CA, USA) for 14 SNPs from 13 unique obesity loci previously identified in populations of European descent [10, 11]. These SNPs included: rs2815752 (*NEGR1*), rs10913469 (*SEC16B*), rs4854344 (*TMEM18*), rs7647305 (*ETV5*), rs10938397 (*GNPDA2*), rs2844479 (*NCR3–AIF1*), rs6265 (*BDNF*), rs10838738 (*MTCH2*), rs7138803 (*BCDIN3D–FAIM2*), rs4788102 (*SH2B1–ATP2A1*), rs6499640 (*FTO*), rs9939609 (*FTO*), rs12970134 (*MC4R*) and rs29941 (*KCTD15*). The genotype distribution of all tested SNPs was in Hardy–Weinberg equilibrium ($p > 10^{-4}$). We obtained successful genotyping call rates of >99.6% for all SNPs and >99.8% for all included samples (across 14 SNPs).

### Statistical analysis

*SNP association analysis* BMI and weight were inverse-normal transformed separately by sex in each panel before association analysis. In addition, in the Fukuoka study panel, we examined the WHR as a variable of fat distribution, which was also inverse-normal transformed. We tested SNPs for the trait association using linear regression analysis in an additive genotype model after adjustment for age classes separately by sex. Age classes were defined according to age distribution in the individual panels, and included ≤40, 41−50, 51−60 and >60 years for the Amagasaki panel, and ≤55, 56−60, 61−65, 66−70 and >70 years for the Fukuoka panel. A one tailed value of $p<0.05$ ($p<0.1$ two-tailed) was considered statistically significant. We combined association results for the two Japanese panels by using the inverse variance method. We used PLINK (version 1.06; http://pngu.mgh.harvard.edu/~purcell/plink/) [29], *R* software (version 2.8.1; www.r-project.org) and rmeta (version 2.16; http://cran.r-project.org) for association test and meta-analysis (websites accessed 6 February 2011).

*Assessment of genetic effect of obesity variants* To assess the proportion of variance for BMI that was explained by each SNP, we calculated a coefficient of determination $R^2$ as: $2f(1-f)\beta^2$, where $f$ is the minor allele frequency and β is the per-allele effect on the standardised values of BMI. We measured the cumulative effect of multiple SNPs by summing the $R^2$ values for individual SNPs.

*Test of ethnic diversity and sex specificity* We compared the per-allele effect size of each SNP on inverse-normal transformed BMI between the ethnic groups (Japanese vs Europeans) and between sexes. Taking into account the well-known male–female differences in body composition (e.g. fat distribution, deposition and accumulation) [30], we tested the potential sex specificity in the genetic associations with obesity. We examined the heterogeneity of the effect size with Cochran's *Q*-test [31].

*Association analysis of type 2 diabetes* While we tested the primary association with obesity measures, we tested type 2 diabetes association as a secondary analysis in the present study. Thus, we performed two-staged analysis for diabetes case–control study. That is, all SNPs were tested for association with type 2 diabetes in stage 1 samples and only SNPs with $p<0.1$ for BMI or weight were then tested in stage 2 samples (ESM Table 1). Using logistic regression analysis, we tested association of candidate SNPs with type 2 diabetes, with and without adjustment for BMI. We adjusted the diabetes trait for sex and BMI, but not for age because age distribution differed between case and control groups; cases were younger than controls, who were defined as being ≥55 years of age.

## Results

### BMI and weight association at reported SNPs

In this study, we tested the association of BMI and weight with 14 SNP loci that had attained genome-wide significance levels ($p<5\times10^{-8}$) in previously performed GWA meta-analyses for obesity in Europeans [10, 11]. In the combined sample ($n$=18,264), we found that 11 of the 14 SNPs had nominally significant ($p<0.1$ two-tailed) associations with BMI and/or weight; these were rs10913469 (*SEC16B*), rs4854344 (*TMEM18*), rs10938397 (*GNPDA2*), rs6265 (*BDNF*), rs10838738 (*MTCH2*), rs7138803 (*BCDIN3D–FAIM2*), rs4788102 (*SH2B1–ATP2A1*), rs6499640 (*FTO*), rs9939609 (*FTO*), rs12970134 (*MC4R*) and rs29941 (*KCTD15*) (Table 2). Furthermore, significant associations between BMI and two SNPs at *FTO* were determined to be independent (i.e. $p=0.016$ for rs6499640 and $p=3.9\times10^{-6}$ for rs9939609, both two-tailed, when these were simultaneously included in the linear regression model). Given the much stronger association for rs9939609 than rs6499640 (Table 2), rs9939609 was considered to be the key SNP for *FTO*, although rs6499640 still appears to have some role. The strength of association did not appear to significantly differ between the study panels (Amagasaki vs Fukuoka), except at *BDNF* ($p<0.01$ for heterogeneity; ESM Table 2). The presence of sexual dimorphism was indicated for two loci, *NEGR1* and *GNPDA2* ($p<0.05$ for heterogeneity; ESM Table 3).

**Table 2** Association of previously reported SNPs with BMI and type 2 diabetes in Japanese individuals

| SNP details | | | | Allele testing | | Obesity measures | | | | Type 2 diabetes (by BMI-adjusted status) | | | | Cases/controls (*n*/*n*) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | BMI (*n*=18,264) | | Weight (*n*=18,264) | | Not adjusted | | Adjusted | | |
| Chr[a] | Neighbouring gene(s) | SNP | Position (B36) | Allele | Frequency | Beta (%) | *p* value | Beta (%) | *p* value | OR | *p* value | OR | *p* value | |
| 1 | *NEGR1* | rs2815752 | 72,585,028 | T | 0.92 | 0.53 | 0.785 | 0.25 | 0.895 | 0.86 | 0.028 | 0.88 | 0.097 | 1,671/6,293 |
| 1 | *SEC16B* | rs10913469 | 176,180,142 | C | 0.23 | 3.00 | 0.014[b] | 2.20 | 0.069 [b] | 1.04 | 0.151 | 1.02 | 0.576 | 6,781/7,307 |
| 2 | *TMEM18* | rs4854344 | 628,144 | A | 0.90 | 8.38 | $7.1\times10^{-7}$[b] | 7.58 | $6.3\times10^{-6}$[b] | 1.18 | $3.2\times10^{-5}$[b] | 1.16 | $2.9\times10^{-4}$[b] | 6,781/7,307 |
| 3 | *ETV5* | rs7647305 | 187,316,984 | C | 0.96 | 0.87 | 0.755 | 0.88 | 0.753 | 1.01 | 0.931 | 1.04 | 0.739 | 1,671/6,293 |
| 4 | *GNPDA2* | rs10938397 | 44,877,284 | G | 0.30 | 4.36 | $1.3\times10^{-4}$[b] | 3.64 | $1.3\times10^{-3}$[b] | 1.07 | 0.007[b] | 1.07 | 0.017[b] | 6,781/7,307 |
| 6 | *NCR3-AIF1* | rs2844479 | 31,680,935 | A | 0.51 | 0.59 | 0.569 | 0.82 | 0.428 | 0.96 | 0.280 | 0.98 | 0.544 | 1,671/6,293 |
| 11 | *BDNF* | rs6265 | 27,636,492 | G | 0.59 | 5.41 | $3.1\times10^{-7}$[b] | 4.21 | $6.3\times10^{-5}$[b] | 1.09 | 0.001[b] | 1.07 | 0.010[b] | 6,781/7,307 |
| 11 | *MTCH2* | rs10838738 | 47,619,625 | G | 0.32 | 3.77 | 0.001[b] | 1.95 | 0.078 [b] | 0.96 | 0.076 | 0.94 | 0.016 | 6,781/7,307 |
| 12 | *BCDIN3D-FAIM2* | rs7138803 | 48,533,735 | A | 0.34 | 1.63 | 0.137 | 1.92 | 0.078 [b] | 1.06 | 0.020[b] | 1.05 | 0.041[b] | 6,781/7,307 |
| 16 | *SH2B1-ATP2A1* | rs4788102 | 28,780,899 | A | 0.14 | 3.52 | 0.018[b] | 3.12 | 0.035 [b] | 1.04 | 0.276 | 1.03 | 0.378 | 6,781/7,307 |
| 16 | *FTO* | rs6499640 | 52,327,178 | A | 0.15 | 3.47 | 0.018[b] | 1.96 | 0.180 | 1.02 | 0.659 | 1.00 | 0.936 | 6,781/7,307 |
| 16 | *FTO* | rs9939609 | 52,378,028 | A | 0.19 | 6.05 | $4.6\times10^{-6}$[b] | 4.81 | $2.5\times10^{-4}$[b] | 1.20 | $4.3\times10^{-10}$[b] | 1.17 | $2.4\times10^{-7}$[b] | 6,781/7,307 |
| 18 | *MC4R* | rs12970134 | 56,035,730 | A | 0.16 | 2.12 | 0.135 | 4.30 | 0.002[b] | 1.11 | 0.002[b] | 1.11 | 0.002[b] | 6,781/7,307 |
| 19 | *KCTD15* | rs29941 | 39,001,372 | C | 0.21 | 0.54 | 0.671 | 2.42 | 0.055[b] | 1.01 | 0.710 | 1.01 | 0.754 | 6,781/7,307 |

Results for Japanese participants from the Amagasaki (*n*=5,695) and Fukuoka (*n*=12,569) panels were combined by meta-analysis; we tested the allele reported to increase BMI in Europeans

Effect sizes are indicated as beta per SD unit of trait (for BMI and weight) and OR (for type 2 diabetes)

The association with type 2 diabetes was tested after adjustment for BMI and sex (see 'Methods' section)

Cohort-wise results are shown in ESM

[a] Chromosome

[b] Suggestive association ($p<0.1$, two-tailed)

## Ethnic heterogeneity in effect sizes

To test for the potential presence of ethnic heterogeneity, we compared the effect sizes (β for BMI) between the Japanese and European populations (Fig. 1a). The sample size was smaller in the Japanese group, and consequently the 95% CI was wider in this group, although the direction of association was concordant between the ethnic groups for all SNPs. However, we did find significant ($p<0.05$) inter-ethnic heterogeneity in per-allele effect at rs2844479 (*NCR3–AIF1*) and rs29941 (*KCTD15*) for standardised BMI, and at rs9939609 (*FTO*) for non-standardised BMI (in kg/m$^2$; ESM Table 4). We calculated $R^2$ as the proportion of phenotypic variance explained by a SNP (see Methods). Based on these $R^2$ measurements, the association of three replicated loci, rs4854344 (*TMEM18*), rs6265 (*BDNF*) and rs10838738 (*MTCH2*), was stronger in the Japanese population than in the population of European descent. We detected no significant gene×gene interactions by regression analysis for BMI associations with 14 SNPs (data not shown); the genotypes of each SNP were concomitantly included assuming an additive effect when combined. For the 14 SNPs, the cumulative $R^2$ totalled 0.65% and 1.2% in the Japanese and Europeans, respectively. Thus, the explained variance tended to be smaller in Japanese than in Europeans.

## Association of obesity susceptibility SNPs with type 2 diabetes

For the case–control study of type 2 diabetes, we genotyped 11 obesity-associated SNPs in the stage 1 and stage 2 panels; the remaining three SNPs were genotyped only in the stage 1 panel (ESM Table 1). Some evidence of association with type 2 diabetes in a direction consistent with the BMI–SNP associations ($p=0.02–4.3\times10^{-10}$ and OR 1.06–1.20 before adjustment for BMI; $p=0.04–2.4\times10^{-7}$ and OR 1.05–1.17 after adjustment for BMI) was provided for six (of 11) SNPs in the Japanese population (Fig. 1b, Table 2). After adjusting for BMI, most of the observed associations between obesity-associated SNPs and diabetes were slightly attenuated, although nominal significance remained (Table 2, ESM Table 5). Considering the possibility of some selection bias in the case–control study design, we also tested the diabetes association by nested case–control comparison within the same population (i.e. Fukuoka panel) and verified a fair consistency in the OR for type 2 diabetes (ESM Table 6). Further, to examine the possibility that the BMI-independent diabetes associations derive from genetic susceptibility to fat distribution, we tested the association with WHR among the control participants in the Fukuoka panel ($n$=4,889), where none of the six SNPs showed significant association with WHR

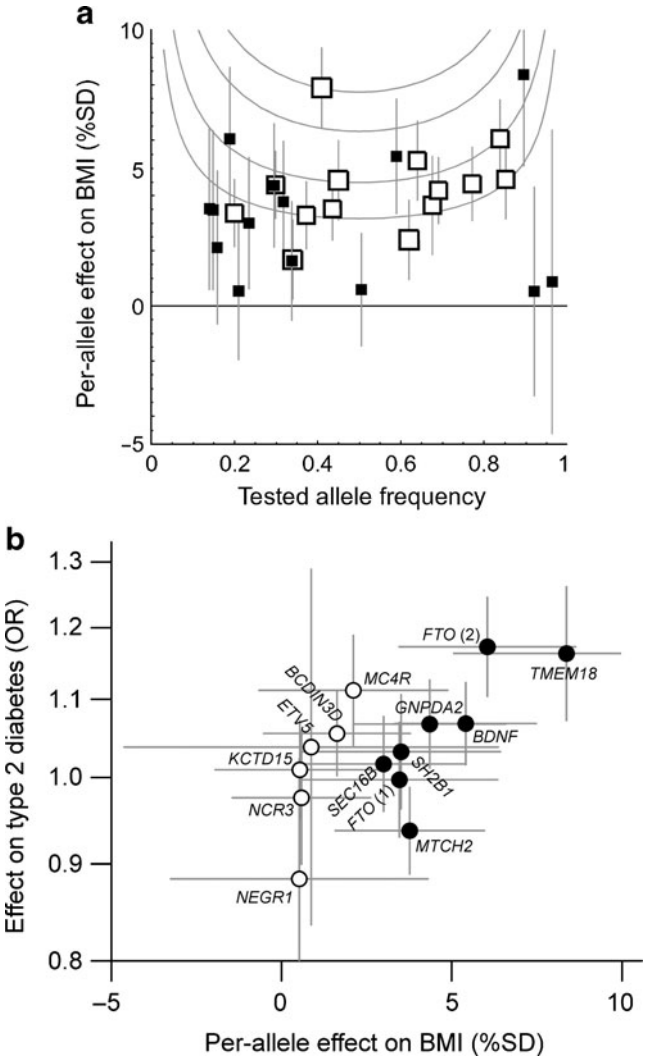


**Fig. 1** Effect size for BMI and type 2 diabetes at SNPs previously reported to be associated with BMI in Europeans. **a** Cross-population comparison of per-allele effect of 14 BMI-associated SNPs between the Japanese and European populations. The per-allele effects (β in % SD) of each variant on BMI are shown by squares (proportional in size to tested sample size) and vertical lines (representing 95% CI) for the Japanese (black squares) and Europeans (white squares). Curves (in grey) indicate $R^2$=0.003, 0.002, 0.001 and 0.0005 (from top to bottom). **b** Comparison of genetic impacts on BMI (β in *x*-axis) and type 2 diabetes adjusted for BMI (OR in *y*-axis) for the 14 SNPs. Those showing significant (black circles) and non-significant (white circles) association with BMI in Japanese are depicted. SNP rs numbers of individual loci are as follows: rs2815752 for *NEGR1*, rs10913469 for *SEC16B*, rs4854344 for *TMEM18*, rs7647305 for *ETV5*, rs10938397 for *GNPDA2*, rs2844479 for *NCR3*, rs6265 for *BDNF*, rs10838738 for *MTCH2*, rs7138803 for *BCDIN3D*, rs4788102 for *SH2B1*, rs6499640 for *FTO*(1), rs9939609 for *FTO*(2), rs12970134 for *MC4R* and rs29941 for *KCTD15*

in a direction consistent with the SNP–diabetes associations (ESM Table 7).

Assuming the potential presence of 'unadjusted' confounding influences of BMI, we plotted (Fig. 2) the data from the genetic studies examining type 2 diabetes

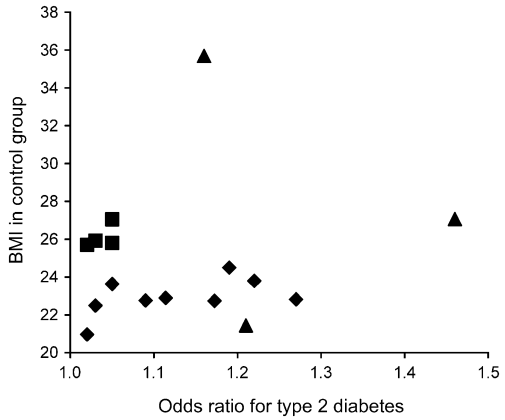


**Fig. 2** Relationship between BMI in the control group (or derived population) and the OR for type 2 diabetes in genetic studies examining type 2 diabetes association at the *FTO* locus (by using rs9939609 or its proxies). The studies are categorised into three ethnic groups: East Asians (diamonds), Europeans (squares) and others (triangles). For further details, see ESM Table 8

association at the *FTO* locus [3, 11, 24, 32–42], our aim being to evaluate the relationship between BMI in the control group (or derived population) and the OR for type 2 diabetes. We detected no apparent relationship between the tested variables ($p$=0.15 for East Asians and $p$=0.41 for Europeans; Fig. 2, ESM Table 8). While there was a significant cross-population difference in the OR for type 2 diabetes (e.g. OR 1.13 and 1.04 for East Asians and Europeans, respectively; $I^2$=89%; $p$=0.003), the BMI-adjusted association with type 2 diabetes proved to be highly significant (OR 1.09, $p=3.0\times10^{-11}$) in the meta-analysis involving 36,064 cases and 61,234 controls of various ethnic origins.

## Discussion

The present study investigated genetic susceptibility to common forms of obesity and its relevance to type 2 diabetes in Japanese populations. Replicating a study of candidate loci previously identified by GWA meta-analyses of Europeans [10, 11], we found some ethnic diversity in obesity variants (Fig. 1a). The top hit associations were consistently reported at *FTO* and *MC4R* in populations of European descent [5, 6, 10–12, 14, 15], whereas equivalent or more pronounced associations with obesity localised to *TMEM18* and *BDNF*, together with modest association at *SEC16B*, *GNPDA2*, *MTCH2*, *BCDIN3D–FAIM2*, *SH2B1–ATP2A1* and *KCTD15*, were found in the Japanese populations (Table 2, ESM Table 4). In addition, we found that the direction of BMI association was concordant between the ethnic groups at all tested loci, although the cumulative effect of the associated SNPs was smaller in Japanese than in Europeans. Of particular note is the fact that, in the present study, we highlighted the genetic impact of six loci on type 2 diabetes, this impact being independent of obesity susceptibility in all six loci.

Recently, two GWA meta-analyses were performed for examination of obesity in populations of European descent; the studies involved >32,000 and >25,000 individuals, with follow-up analysis using genotypes from large cohorts (>50,000 samples in total) and computer-generated association results [10, 11]. Collectively, these studies revealed 13 unique obesity-associated loci in Europeans. Four studies in total, including our present study, have attempted to replicate the obesity association in East Asians; of these, two studies involved Japanese individuals and two involved Chinese individuals [23–25]. While the design of these studies was not identical (two case–control studies, one quantitative trait analysis and one study with both-types of analytical approaches combined), all four studies consistently reported significant association with obesity at the *GNPDA2* (rs10938397) and *FTO* (rs9939609 or its proxies) loci in East Asians. The present study, which is the largest of the four studies and the only one that involved quantitative trait analysis using the general population sample, has enabled us to validate nine other SNP loci. Eight of these SNP loci had previously shown obesity association in one or two studies [23–25], whereas replication at *MTCH2* (rs10838738) has not been previously reported in East Asians.

Compared with the data for Europeans [10, 11], the variance for BMI explained by individual SNP loci appeared to be modest as a whole in the Japanese population, except for *TMEM18* rs4854344 ($R^2$=0.13%), *BDNF* rs6265 ($R^2$=0.1%) and *MTCH2* rs10838738 ($R^2$= 0.06%; Fig. 1b, ESM Table 4). It should be kept in mind that the larger cumulative effect on BMI in Europeans than in Japanese is partly due to the 'winner's curse effect' [43], where the original meta-analysis tends to overestimate the true population effect, in addition to ethnic difference in at-risk alleles. We found that the effect size (β) was larger at *TMEM18* rs4854344 than at *FTO* rs9939609 in the independent panels of Japanese samples (ESM Table 2). Nevertheless, there seems to be an overall consistency of genetic variants for susceptibility to obesity between the examined ethnic groups.

A remaining issue of interest is whether the correlations between obesity-associated SNPs and type 2 diabetes can be explained by the SNP–trait and trait–type 2 diabetes associations [18]. A simple way to assess the causal direction of associations (i.e. whether increased adiposity resulting from the variant under investigation is causally related to type 2 diabetes) is to test the inconsistency of the SNP–diabetes association both with and without adjustment for BMI. In this regard, our results, although not conclusive at some loci, indicated

that six obesity variants could lead to altered fat mass and altered susceptibility to type 2 diabetes, at least in part, through separate mechanisms, based on the statistical evidence for independence following analysis of either the whole sample or separately by sex (Table 2, ESM Table 5). Interestingly, a previous study in Europeans [18] demonstrated that the associations between *FTO* variants and obesity-related metabolic traits (including type 2 diabetes) are likely to be entirely mediated by BMI; this study also emphasised that the use of appropriately powered studies was important in making such assessments. Our meta-analysis for the *FTO* locus, involving 36,064 cases and 61,234 controls, has shown that the BMI-adjusted diabetes association is highly significant (OR 1.09, $p=3.0\times10^{-11}$). Based on analysis of stratification of Japanese samples by extent of obesity (underweight, normal weight, overweight and obese), sex (men, women) and age (<60 and ≥60 years), we determined that the strength of type 2 diabetes association tended to be more pronounced among underweight people aged <60 years in both sexes, although it did not reach statistical significance due to relatively small sample size ($n<100$ for cases and controls) in the corresponding (age- and obesity-stratified) group ($p=0.18$ for a group of underweight people aged <60 years vs the others; Fig. 3, ESM Fig. 1). Previous epidemiological studies have reported that being underweight is likely to be associated with the risk of type 2 diabetes in the Japanese population [44, 45] and that Japanese people have low insulin secretory capacity and a high risk of type 2 diabetes at BMIs lower than the existing WHO cut-off point for being overweight, 25 kg/m$^2$ [46, 47]. Thus, together with other, unidentified confounding factors, the extent of obesity (>2/3 of the Japanese participants were underweight or normal weight) may account for cross- and within-population differences in type 2 diabetes susceptibility at obesity loci such as *FTO*. Despite limited data availability at present on the topic, the direction of BMI-adjusted diabetes association seems to be concordant in some five studies [3, 4, 10, 24, 42] that have investigated the 14 obesity variants (ESM Table 9).

Sex specificity is another issue of interest in the genetics of obesity. Some epidemiological studies have suggested that different genes influence variation of BMI in men and women [30]. However, little evidence has been provided to support this notion. In the present study, we found nominally significant sex-related differences in the effect sizes for two obesity variant loci, *NEGR1* and *GNPDA2* (ESM Table 3). Although heterogeneity was not statistically significant ($p=0.09$), obesity association only in women appeared to be replicated for *MC4R*. Notably, a previous study has reported the equivalent type of sexual dimorphism for the association between a *MC4R* variant and obesity measures in the Swedish population [42]. Despite no prior evidence of sex specificity at this locus in Europeans [4, 48], these findings warrant further investigation with reference to ethnic homogeneity and/or the specificity of target populations.

We acknowledge several limitations inherent in the present study. Specifically, the sample size used to screen obesity association was smaller for the Japanese group ($n=18,264$) than that used for the European GWA meta-analyses ($n>25,000$ in the discovery stage). Therefore, we assessed statistical power for each of the tested loci, assuming effect sizes equivalent to those reported in the original GWA studies for the allele frequencies in the Japanese (ESM Table 10). Apart from the replicated

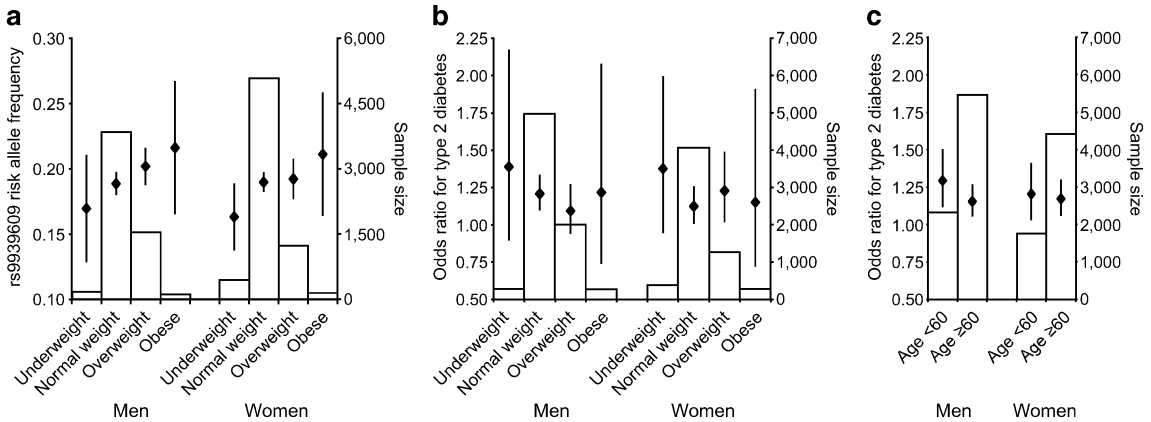


**Fig. 3** Stratification of Japanese sample by extent of obesity, sex (men and women), and age (<60 and ≥60 years) in relation with genetic impacts at *FTO* rs9939609. Obesity was graded as: underweight (BMI <18.5 kg/m$^2$), normal weight (18.50–24.99 kg/m$^2$), overweight (25.00–29.99 kg/m$^2$) and obese (≥30 kg/m$^2$). The samples were analysed separately by sex. **a** Risk allele (A in the plus strand) frequencies of rs9939609, compared among four subgroups of Fukuoka panel ($n=12,569$) stratified by extent of obesity. **b** ORs for type 2 diabetes, compared among four subgroups of case–control sample (6,781 cases, 7,307 controls) stratified by extent of obesity. **c** ORs for type 2 diabetes, compared between two subgroups of case–control sample (6,781 cases, 7,307 controls) stratified by age. Whiskers indicate 95% CIs. For detailed stratification, see ESM Fig. 1

associations, sufficient power (i.e. >0.8) was not attainable for two (of 14) SNP loci in the Japanese samples. In addition to the issue of power, differences in linkage disequilibrium patterns between the ethnic groups could also have contributed to the lack of replicated association at a given locus. We found some cross-population differences in linkage disequilibrium relations at several SNP loci, which could also attenuate (or strengthen) the association signals accordingly. Regional examination of SNP–obesity association, which is ongoing in Asians, but not point-wise studies, will resolve this issue.

In summary, we confirmed that 11 SNPs from ten candidate loci were significantly associated with BMI in Japanese individuals, as was previously reported in Europeans. We also found some cross-population differences in the effect sizes of individual obesity variants. These studies, moreover, highlight the genetic influences on type 2 diabetes that appear to be independent of BMI, as well as the potential presence of sex specificity in the genetics of common obesity.

**Acknowledgements** This work was supported by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation Organization (NIBIO), the Manpei Suzuki Diabetes Foundation, a grant from National Center for Global Health and Medicine (NCGM) and by the Ministry of Education, Cultures, Sports, Science and Technology, all of Japan. We acknowledge the outstanding contributions of the employees of NCGM, who provided technical and infrastructural support for this work. Above all, we thank the patients and study participants who made this work possible and who gave it value. We thank all the people who continuously support the Hospital-based Cohort Study at NCGM, the Amagasaki Study and the Kyushu University Fukuoka Cohort Study in Japan. We also thank S. Kono, M. Ogasawara, C. Makibayashi and the many physicians of the Amagasaki Medical Association for their contribution in collecting DNA samples and accompanying clinical information. Part of the DNA samples from type 2 diabetes cases used for this research were provided from the Leading Project for Personalized Medicine at the Ministry of Education, Culture, Sports, Science and Technology, Japan.

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

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