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厚生労働科学研究費補助金

創薬基盤推進研究事業

創薬および臨床試験の効率化に資するメタボリックシンドロームと
心血管病のモデルラットの開発研究

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研究代表者 加藤 規弘

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目 次

I. 総括研究報告	
創薬および臨床試験の効率化に資するメタボリックシンドロームと 心血管病のモデルラットの開発研究	----- 1
加藤 規弘	
II. 分担研究報告	
1. メタボリックシンドロームと心血管病のモデルラットの開発研究	--- 3
加藤 規弘	
2. 心血管病評価用ラットパネルの整備に関する研究	----- 5
並河 徹	
3. モデルラットを用いた心血管病の成因・病態に関する研究	----- 7
福田 昇	
III. 研究成果の刊行に関する一覧表	----- 9
IV. 研究成果の刊行物・別刷	----- 10

厚生労働科学研究費補助金（創薬基盤推進 研究事業）
総括研究報告書

創薬および臨床試験の効率化に資するメタボリックシンドロームと
心血管病のモデルラットの開発研究

研究代表者 加藤 規弘 国立国際医療研究センター部長

研究要旨 心血管病リスクファクターである高血圧や糖尿病に関しては、各々適切なモデル動物がいくつか存在するが、心血管病そのものに関しては、必ずしも十分な整備状況といえないため、新規モデルの開発を試みる。高血圧自然発症ラット（SHR）系統から新規に作成した10系統のうち、「劇症型脳卒中易発症 SHR」は顕著な脳卒中易発症を示した。また、げっ歯類の弱点とされてきた cholesterol ester transfer protein（CETP）のトランスジェニック・ラットの作成に成功し、ヒトでの動脈硬化型脂質異常が認められた。SHRの病態解析により、補体C3の遺伝的な発現増加と、間葉系細胞の合成型形質転換による心血管リモデリングへの関与を認めた。

研究分担者

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A. 研究目的

本研究は（1）ヒトの動脈硬化を模する上でのげっ歯類の弱点とされてきた cholesterol ester transfer protein（CETP）の欠損を補うべく、トランスジェニック（Tg）ラットを作成して、脂質代謝障害から動脈硬化への進展、特に心筋梗塞の新規モデル動物を開発すること、（2）メタボリックシンドロームなどのリスクファクターの集積に関して、代表的な組み合わせパターン疾患モデルラットを新たに開発すること、そして（3）これらを創薬および臨床試験の効率化に役立てるべく“心血管病評価用ラットパネル”として整備すること、を主たる目的とする。

B. 研究方法

本研究の実施計画は大きく三つの部分から成る。一つ目は、SHRにヒトCETPを導入したTgラットを作成する部分であり、二つ目は、同Tgラットも含めて、心血管病関連形質（リスクファクター形質と個々の臓器障害）を特徴的に有する近交系ラット群から“心血管病評価用ラットパネル”を作成する部分であり、三つ目は心血管病の病態探究である。

（倫理面への配慮）

当該研究ではヒト由来試料を取り扱わない。「研究機関等における動物実験等の実施に関する基本指針」および「厚生労働省における動物実験等の実施に関する基本指針」を遵守し、動物愛護に配慮して研究立案し、立案した動物実験計画の妥当性について、機関内の動物実験委員会の審査と許可を受けて進める。

C. 研究結果

1. ヒトCETPを導入したTgラットの作成

transgeneの導入に成功した系統が全部で5系統あり、そのうちの、3系統の繁殖・維持に成功した。血中脂質レベルに関しては、導入されたcopy数に応じて（多くなればなるほど）総コレステロール（TC）が顕著に低下し、逆に中性脂肪（TG）が顕著に上昇していた〔予備の実験データでは、wild-typeに比べてTCが半分、TGが約4倍に増加〕。ラットTCの最大アポ蛋白分画はHDLであることから、HDL↓、TG↑という、ヒトでの動脈硬化型脂質異常が見られた。

2.“心血管病評価用ラットパネル”の作成

初年度に新規作成した10系統のなかでも特に顕著な病態を示す「劇症型脳卒中易発症SHR（E-SHRSP）」の脳卒中発症に関する臨床的評価を行った。E-SHRSPは、通常餌での

飼育下で高率に脳卒中を自然発症すること、食塩負荷により更に発症時期を早められること、生後 10-12 週という幼若期の血圧の比較的マイルドな (5-10 mm Hg) 減少が脳卒中発症抑制に相当な影響を及ぼす可能性が高いこと、等が判明した。

3. モデルラットでの心血管病の病態探究

心血管病のなかでも特に腎障害に対して、血圧影響の無い培養系において補体 C3 の病因/病態的関与を検討した。WKY、SHR、SHR-SP の大動脈および腎系球体から血管平滑筋細胞、腎メサンジウム細胞を単離継代培養し、それらに対する C3a 受容体拮抗薬、pre-pro-C3 アンチセンスオリゴの影響を評価した。C3a 受容体拮抗薬、C3 アンチセンスオリゴは共に Ang II 産生等を抑制した。

D. 考察

CETP-Tg ラットに関しては、今後、更に兄妹交配を重ねてホモ化を進めるとともに、食餌負荷実験での phenotype、病理学的所見を調べていく。E-SHRSP は、脳卒中の自然歴と薬物介入の timing を検討するうえで有用性が高いモデルと考えられる。

従来、高血圧性心血管/腎障害は高血圧の結果と考えられていたが、血圧影響の無い培養系においても、内因性異常として C3 の遺伝的過剰発現が考えられた。

E. 結論

CETP の Tg ラットに関して phenotype の評価を開始し、ヒトでの動脈硬化型脂質異常が認められた。高血圧性腎障害の病態改善に、補体 C3 阻害の治療的有用性を確認した。

F. 健康危険情報

特記すべきものなし

G. 研究発表

1. 論文発表

Takeuchi F, Kato N, *et al.* Association of obesity susceptibility genetic variants with type 2 diabetes in the Japanese. *Diabetologia*. 2011,

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Takeuchi F, Kato N, *et al.* Detection of common single nucleotide polymorphisms synthesizing quantitative trait association of rarer causal variants. *Genome Res*. 2011 May 16. [Epub ahead of print]

Takeuchi F, Kato N, *et al.* Confirmation of ALDH2 as a major locus of drinking behavior and of its variants regulating multiple metabolic phenotypes in Japanese. *Circ J*. 2011, 75(4):911-8.

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H. 知的財産権の出願・登録状況

特記すべきものなし。

厚生労働科学研究費補助金（創薬基盤推進 研究事業）
分担研究報告書

メタボリックシンドロームと心血管病のモデルラットの開発研究

研究代表者 加藤 規弘 国立国際医療研究センター部長

研究要旨 心血管病リスクファクターである高血圧や糖尿病に関しては、各々適切なモデル動物がいくつか存在するが、心血管病そのものに関しては、必ずしも十分な整備状況といえないため、新規モデルの開発を試みる。高血圧自然発症ラット（SHR）系統から作成した「劇症型脳卒中易発症 SHR」は顕著な脳卒中易発症性を示す。また、げっ歯類の弱点とされてきた cholesterol ester transfer protein（CETP）のトランスジェニック・ラットを作成し、総コレステロール（TC）が顕著に低下し、逆に中性脂肪（TG）が顕著に上昇していた。

A. 研究目的

本研究は(1)ヒトの動脈硬化を模する上でのげっ歯類の弱点とされてきた cholesterol ester transfer protein（CETP）の欠損を補うべく、トランスジェニック（Tg）ラットを作成して、脂質代謝障害から動脈硬化への進展、特に心筋梗塞の新規モデル動物を開発すること、(2)メタボリックシンドロームなどのリスクファクターの集積に関して、代表的な組み合わせパターンの疾患モデルラットを新たに開発すること、そして(3)これらを創薬および臨床試験の効率化に役立てるべく“心血管病評価用ラットパネル”として整備すること、を目的とする。

B. 研究方法

本研究の実施計画は大きく二つの部分から成る。一つは、SHRにヒトCETPを導入したTgラットを作成する部分であり、もう一つは、同Tgラットも含めて、心血管病関連形質（リスクファクター形質と個々の臓器障害）を特徴的に有する近交系ラット群から“心血管病評価用ラットパネル”を作成する部分である。

（倫理面への配慮）

当該研究ではヒト由来試料を取り扱わない。「研究機関等における動物実験等の実施に関する基本指針」および「厚生労働省における動物実験等の実施に関する基本指

針」を遵守し、動物愛護に配慮して研究立案し、立案した動物実験計画の妥当性について機関内の動物実験委員会の審査と許可を受けて進める。

C. 研究結果

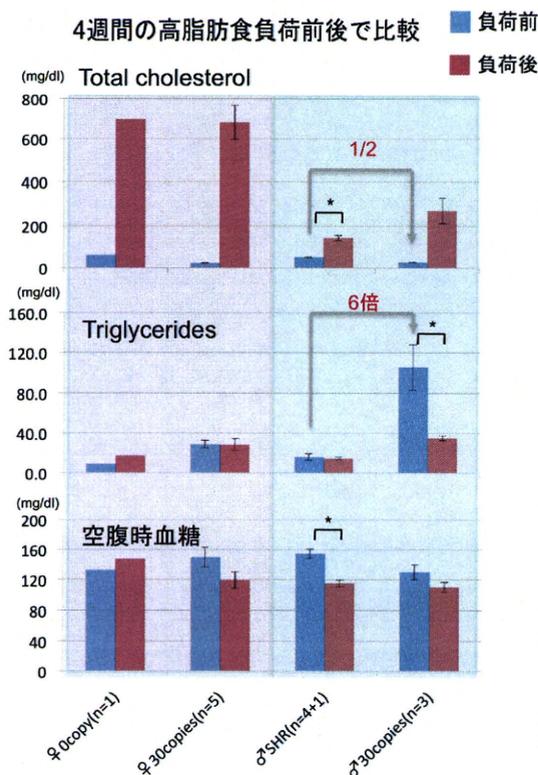
1. ヒトCETPを導入したTgラットの作成
transgene の導入に成功した系統が全部で5系統あり、そのうちの、3系統の繁殖・維持に成功した。各系統の導入 copy 数は、3、10、30の3種類であり、血中脂質レベルに関しては、導入された copy 数に応じて（多くなればなるほど）総コレステロール（TC）が顕著に低下し、逆に中性脂肪（TG）が顕著に上昇していた〔予備的実験データでは、wild-type に比べてTCが半分、TGが約4倍に増加〕。ラットTCの最大アポ蛋白分画はHDLであることから、HDL↓、TG↑という、ヒトでの動脈硬化型脂質異常が見られた。

2. “心血管病評価用ラットパネル”の作成
初年度に新規作成した10系統のなかでも特に顕著な病態を示す「劇症型脳卒中易発症 SHR（E-SHRSP）」の脳卒中発症に関する臨床的評価を行った。E-SHRSPは、通常餌での飼育下で5ヶ月齢までに雄87%、雌54%が脳卒中を自然発症すること、食塩負荷により更に発症時期を早められること、生後10-12週という幼若期の血圧の比較的

マイルドな (5-10 mm Hg) 減少が脳卒中発症抑制に相当な影響を及ぼす可能性が高い (従って、前高血圧段階での降圧治療が有効である) こと、等が判明した。

D. 考察

CETP-Tg ラットに関しては、今後、更に兄妹交配を重ねてホモ化を進めるとともに、食餌負荷実験での phenotype、病理学的所見を調べていく (下図参照)。



E-SHRSP は、脳卒中の自然歴と薬物介入の timing を検討するうえで有用性が高いと考えられる。

E. 結論

CETP のトランスジェニック・ラットに関しては、3系統の繁殖・維持に成功した。血中脂質レベルは、導入された copy 数に応じて総コレステロールが顕著に低下し、逆に中性脂肪 (TG) が顕著に上昇していた。

F. 研究発表

論文発表

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Takeuchi F, Kato N et al. Confirmation of ALDH2 as a major locus of drinking behavior and of its variants regulating multiple metabolic phenotypes in Japanese. *Circ J.* 2011, 75(4):911-8.

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Takeuchi F, Kato N et al. Evaluation of pharmacogenetic algorithm for warfarin dose requirements in Japanese patients. *Circ J.* 2010, 74:977-82.

G. 知的財産権の出願・登録状況

特記すべきものなし。

厚生労働科学研究費補助金（創薬基盤推進 研究事業）
分担研究報告書

心血管病評価用ラットパネルの整備に関する研究

研究分担者 並河 徹 島根大学医学部 教授

研究要旨 複数の遺伝子領域の相互作用により高血圧、脳卒中を起こす double & triple congenic 系統の作出を行った。

A. 研究目的

近年の大規模ゲノムワイド関連解析により同定された原因遺伝子の効果は非常にわずかであることがわかってきた。このことは、遺伝子間の相互作用が疾病発症の鍵となることを示唆する。そこで、本研究では、遺伝的高血圧モデルから、複数の素因遺伝子領域を正常血圧モデルに導入することで、限られた数の領域（遺伝子座）の相互作用により親系統と同等の高血圧や脳卒中を起こす新たな系統を作出し、遺伝子間相互作用の解明を行い、新たな発症機序に基づいて創薬につなげることを目的とした。

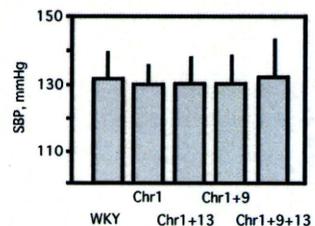
B. 研究方法

ラットの遺伝解析から血圧や脳卒中発症に関与することが示唆された染色体領域を、遺伝的マーカーをスクリーニングしながら5-6世代戻し交配することによって、正常血圧ラットに導入していく。さらに異なる領域を導入した系統同士を交配することで、2カ所以上の領域を入れ替えたコンジュニック系統を作り、その表現形質を確認する。

C. 研究結果

- ① 脳卒中関連遺伝子領域：脳卒中感受性遺伝子領域2カ所を脳卒中易発症ラット（SHRSP）から脳卒中抵抗性ラット（SHR）に導入した double congenic rat を完成し、この2領域が相乗的に脳卒中を起こしやすくすることを確認した。
- ② 高血圧関連遺伝子領域：遺伝解析によって

同定された高血圧関連遺伝子領域を3カ所（染色体1, 9, 13のそれぞれ一部）、高血圧ラット（SHRSP）から正常血圧ラット（WKY）に移した triple congenic rat を作成し、その血圧への効果を検討したが、有意な血圧上昇は確認できなかった（図）。



D. 考察

ヒトにおける大規模ゲノム解析から、生活習慣病の発症には複数の遺伝素因の相互作用が重要である可能性が指摘されている。このことは、動物実験において、一カ所の高血圧原因遺伝子領域を正常血圧ラットに導入するのみでは高血圧を起こし得ないことから支持される。本研究では、SHRSPの脳卒中感受性に関しては、2つの染色体領域（全ゲノムのおよそ3%に相当）によってそのほとんどが説明できること、2領域は相乗的な効果を有することが明らかとなった。これに比べて血圧については、より多くの遺伝子領域が複雑な相互作用をしている可能性が示唆された。脳卒中感受性遺伝子領域に関する double congenic 系統のようなモデルは、今後遺伝子間相互作用を解明するためのツールとして有用と考える。

E. 結論

有限の遺伝子領域の組み合わせで多因子

遺伝性疾患のモデル動物を構築することができることを示した。このようなモデルを複数用意することは、単独では効果の小さい遺伝的変異の相互作用に基づく生活習慣病の発症メカニズムの解明に役立て得る。

F. 研究発表

1. 論文発表

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Blood pressure-independent factors determine the susceptibility to delayed neuronal death in the stroke-prone spontaneously hypertensive rats. *Cell Mol Neurobiol.* 2010;30(2):283-7.

2. 学会発表

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Xiao B, Nabika T: Effect of a 1.8-Mbp genomic fragment on chromosome 1 on sympathetic response to stressors: Evaluation in reciprocal congenic strains between SHRSP and WKY. The X VIII th International Workshop on Genetic Systems in the Rat. November 30-December 3, 2010. Kyoto, Japan.

並河 徹: ラット第1染色体上の1.5Mbpの領域が SHRSPのストレス反応性に関与する. 第33回日本高血圧学会総会, 福岡, 2010年10月15~17日.

Xiao B, Nabika T: Effect of a small genomic fragment on chromosome 1 on sympathetic response to stressors: Evaluation in reciprocal congenic strains between SHRSP and WKY. 6th International ISP Congress and 14th International SHR Symposium. September 22-25, 2010. Montreal, Canada.

Xiao B, Nabika T: Effects of small genomic fragment on chromosome 1 on the sympathetic reactivity to stresses: Evaluation in the reciprocal congenic strains between WKY and SHRSP. 第57回日本実験動物学会総会. 京都市 2010年5月12日~14日

崔 宗虎, 並河 徹: SHRSP/IzmとSHR/Izmのコンジェニックラットによる脳卒中関連遺伝子の探索, 第57回日本実験動物学会総会, 京都市 2010年5月12日~14日

G. 知的財産権の出願・登録状況 特記すべきものなし。

厚生労働科学研究費補助金（創薬基盤推進 研究事業）
分担研究報告書

モデルラットを用いた心血管病の成因・病態に関する研究

研究分担者 福田 昇 日本大学大学院総合科学研究科教授

研究要旨 高血圧のモデルラットである高血圧自然発症ラット（SHR）では補体 C3 を遺伝的に発現し、間葉系細胞の合成型形質転換により心血管リモデリングに関与している。

A. 研究目的

高血圧自然発症ラット(SHR)由来血管平滑筋細胞(VSMC)は血圧と関係なく合成型に形質変換し、アンジオテンシン(Ang)IIを酸性し、過剰増殖を示す。我々は、SHR由来間葉系細胞はWKYに比較して補体C3を発現し、この補体C3が心血管病を惹起している可能性を報告してきた。また補体C3は転写因子KLF-5の発現増加を介して、間葉系細胞を合成型形質に転換している事を報告した。今回SHR、SHR-SP由来VSMCおよび腎メサンジウム細胞(MC)の形質変換、過剰増殖、Ang II産生における補体C3の関与を検討した。

B. 研究方法

WKY、SHR、SHR-SPの大動脈および腎糸球体からVSMC、MCを単離継代培養し、DNA合成、形質マーカー、Ang II産生を評価した。これらに対するC3a受容体拮抗薬、pre-pro-C3に対するアンチセンスオリゴの作用を検討した。*in vivo*で食塩負荷WKY、SHR-SPにおけるBrdUの取り込みで臓器増殖を評価し、C3a受容体拮抗薬の作用を検討した。

(倫理面への配慮)

日本大学動物実験指針に基づき、動物実験を行った。

C. 研究結果

SHR、SHR-SP由来MCおよびVSMCは、WKY由来のMCおよびVSMCに比して過剰増殖を示し、KLF-5、合成型形質マーカーを

高発現し、Ang IIを過剰に産生していた。C3a受容体拮抗薬、C3アンチセンスオリゴはSHR、SHR-SP由来MCおよびVSMCの過剰増殖、KLF-5、合成型形質マーカー発現、Ang II産生を抑制した。*in vivo*ではWKYに比して、SHR-SP腎臓でBrdUの取り込みは亢進しており、C3a受容体拮抗薬の全身投与により抑制された。

D. 考察

高血圧性心血管/腎障害は高血圧の結果と考えられていたが、血圧影響の無い培養系においても、SHRおよびSHR-SP由来MCやVSMCの過剰増殖が観られ、高血圧の結果ではなく、内因性異常としてC3の遺伝的過剰発現が考えられた。C3は最終的にC3aとなる。C3aは免疫系に関与しているが、アナフィラトキシンとしてVSMCの増殖にも関与している。またC3は間葉系細胞の脱分化を維持している事が報告され、今回の結果からSHRおよびSHR-SPのMCやVSMCではC3aが合成型形質変換、Ang II産生に関与していると考えられた。

E. 結論

高血圧のモデルラットであるSHRおよびSHR-SPでは遺伝的に心血管/腎臓などの間葉系臓器で補体C3が過剰に発現し、間葉系細胞を合成型に形質変換し、Ang II産生により心血管/腎臓のリモデリングに関与している。したがって高血圧性血管障害、腎硬化症などの病態における補体C3の関与が考えられ、新たな高血圧臓器合併症の治療ター

ゲットになると考えられた。

F. 研究発表

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G. 知的財産権の出願・登録状況
特記すべきものなし。

別添5

研究成果の刊行に関する一覧表

【書籍】

著者氏名	論文タイトル名	書籍全体の編集者名	書籍名	出版社名	出版地	出版年	ページ
加藤規弘	高血圧のゲノムワイド関連解析		Annual Review of Circulation 2011	中外医学社	東京	2011	94-99
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【雑誌】

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Takeuchi F, Kato N <i>et al.</i>	Association of obesity susceptibility genetic variants with type 2 diabetes in the Japanese.	<i>Diabetologia</i>	54(6)	1350-1359	2011
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Association of genetic variants for susceptibility to obesity with type 2 diabetes in Japanese individuals

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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.

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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.

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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 ($p = 7.1 \times 10^{-7}$ for BMI). Among the 11 SNPs showing significant obesity association, six were also associated with diabetes (OR 1.05–1.17; $p = 0.04$ – 2.4×10^{-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; $p = 7.8 \times 10^{-10}$) 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 (<i>n</i>)	5,695	12,569
Women (<i>n</i>)	2,290	6,898
Men (<i>n</i>)	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} ≥7.0 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} ≤5.0%; 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 ($p < 5 \times 10^{-8}$) 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 $p = 3.9 \times 10^{-6}$ 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/h)					
Chr ^a	Neighbouring gene(s)	SNP	Position (B36)	Allele	Frequency	BMI (n=18,264)		Type 2 diabetes		Adjusted				
						Beta (%)	p value	OR	p value					
1	<i>NEGR1</i>	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	<i>SEC16B</i>	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	<i>TMEM18</i>	rs4854344	628,144	A	0.90	8.38	7.1 × 10 ^{-7b}	7.58	6.3 × 10 ^{-6b}	1.18	3.2 × 10 ^{-5b}	1.16	2.9 × 10 ^{-4b}	6,781/7,307
3	<i>ETV5</i>	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	<i>GMPDA2</i>	rs10938397	44,877,284	G	0.30	4.36	1.3 × 10 ^{-4b}	3.64	1.3 × 10 ^{-3b}	1.07	0.007 ^b	1.07	0.017 ^b	6,781/7,307
6	<i>NCR3-AIF1</i>	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	<i>BDNF</i>	rs6265	27,636,492	G	0.59	5.41	3.1 × 10 ^{-7b}	4.21	6.3 × 10 ^{-5b}	1.09	0.001 ^b	1.07	0.010 ^b	6,781/7,307
11	<i>MTCH2</i>	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	<i>BCDIN3D-FAIM2</i>	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	<i>SH2B1-ATP2A1</i>	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	<i>FTO</i>	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	<i>FTO</i>	rs9939609	52,378,028	A	0.19	6.05	4.6 × 10 ^{-6b}	4.81	2.5 × 10 ^{-4b}	1.20	4.3 × 10 ^{-10b}	1.17	2.4 × 10 ^{-7b}	6,781/7,307
18	<i>MC4R</i>	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	<i>KCTD15</i>	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

^aChromosome

^bSuggestive 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 \times 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 ($p = 0.02\text{--}4.3 \times 10^{-10}$ and OR 1.06–1.20 before adjustment for BMI; $p = 0.04\text{--}2.4 \times 10^{-7}$ 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

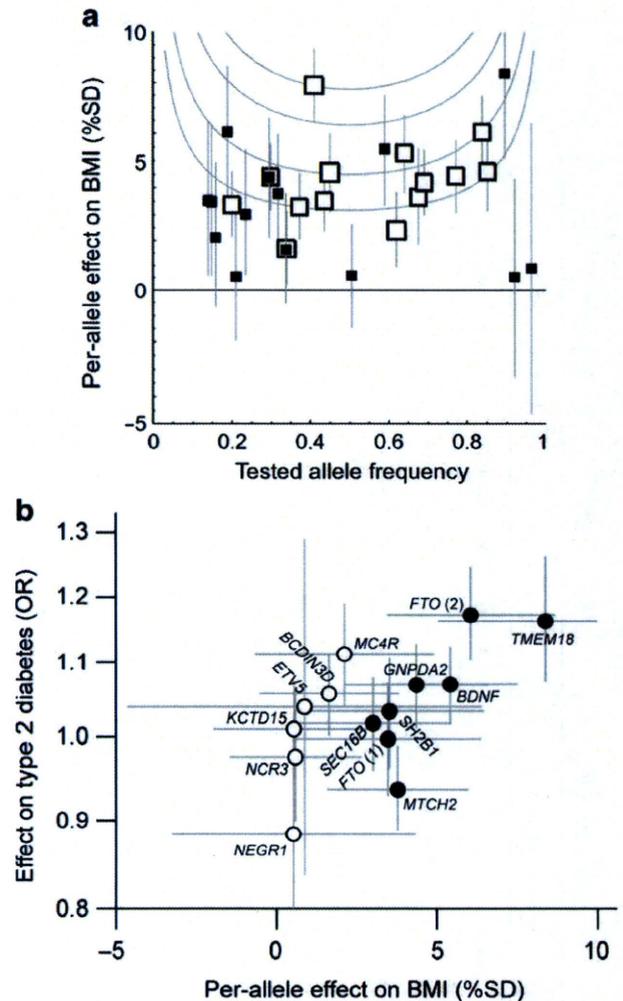


Fig. 1 Effect size for BMI and type 2 diabetes at SNPs previously reported to be associated with BMI in Europeans. **a** Cross-population comparison of per-allele effect of 14 BMI-associated SNPs between the Japanese and European populations. The per-allele effects (β in %SD) of each variant on BMI are 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

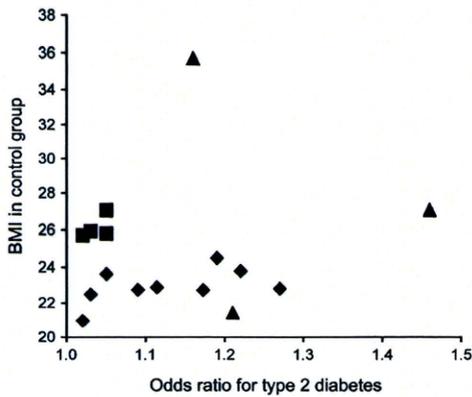


Fig. 2 Relationship between BMI in the control group (or derived population) and the OR for type 2 diabetes in genetic studies examining type 2 diabetes association at the *FTO* locus (by using rs9939609 or its proxies). The studies are categorised into three ethnic groups: East Asians (diamonds), Europeans (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, $p=3.0 \times 10^{-11}$) 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, $p=3.0 \times 10^{-11}$). 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/m² [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

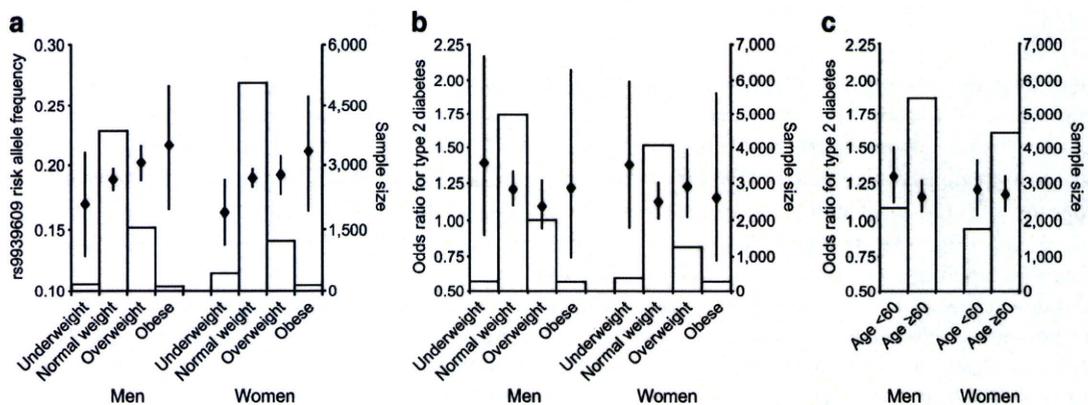


Fig. 3 Stratification of Japanese sample by extent of obesity, sex (men and women), and age (<60 and ≥60 years) in relation with genetic impacts at *FTO* rs9939609. Obesity was graded as: underweight (BMI <18.5 kg/m²), normal weight (18.50–24.99 kg/m²), overweight (25.00–29.99 kg/m²) and obese (≥30 kg/m²). 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.

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

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