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REVIEW

Cell transplantation therapy for diabetes mellitus: endocrine pancreas and adipocyte

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Abstract. Experimental transplantation of endocrine tissues has led to significant advances in our understanding of endocrinology and metabolism. Endocrine cell transplantation therapy is expected to be applied to the treatment of metabolic endocriopathies. Restoration of functional pancreatic beta-cell mass or of functional adipose mass are reasonable treatment approaches for patients with diabetes or lipodystrophy, respectively. Human induced pluripotent stem (iPS) cell research is having a great impact on life sciences. Doctors Takahashi and Yamanaka discovered that the forced expression of a set of genes can convert mouse and human somatic cells into a pluripotent state [1, 2]. These iPS cells can differentiate into a variety of cell types. Therefore, iPS cells from patients may be a potential cell source for autologous cell replacement therapy. This review briefly summarizes the current knowledge about transplantation therapy for diabetes mellitus, the development of the endocrine pancreas and adipocytes, and endocrine-metabolic disease-specific iPS cells.

Key words: Transplantation, Diabetes mellitus, Pancreas, Adipocyte, Development

Transplantation in endocrinology

In 1683, Conrad Brunner (1653-1727) removed the pancreas from a dog and noted the resulting polydipsia and polyuria, two hallmark symptoms of diabetes [3]. In 1889, Oscar Minkowski (1858-1931) transplanted canine pancreases into pancreatectomized dogs and noticed a decrease in the severity in the symptoms. This experiment demonstrated that the pancreas is responsible for the regulation of blood glucose [4]. The first recorded human pancreatic xenotransplantation was performed in 1893. Minkowski transplanted pieces of freshly slaughtered sheep's pancreas into a diabetic 15-year-old boy; however, the boy died three days after the operation. Concern about the detrimental effects of exocrine acinar cells on graft function and viability has stimulated the development of islet cell isolation and transplantation procedures.

In addition to research on diabetes mellitus, transplantation experiments involving other tissues and organs have increased our understanding of endocrinology. In 1849, Adolph Berthold (1803-1861) reported on the transplantation of testes in cockerels [3]. He transplanted testes from young cockerels into the abdominal cavity of the castrated cockerels and found that the recipients continued to retain the normal secondary sexual characteristics. Moritz Schiff (1823-1896) showed that intra-abdominal transplantation of the thyroid gland could prevent the fatal results of total thyroidectomy [5]. George Murray (1865-1939) provided the first account of a human patient with myxedema given substitution with subcutaneous thyroid tissues, which had a beneficial effect [6]. Early in the 20th century, several investigators showed that transplantation of the parathyroid gland prevented the development of neuromuscular symptoms in parathyroidectomized animals [7].

These studies suggested that endocrine cells may be suitable for transplantation, because they are individual functional units that sense extracellular stimuli and secrete hormones.

Pancreas and islet transplantation in diabetes mellitus

The pancreas plays a key role in the maintenance of nutritional homeostasis through its exocrine and endo-

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crine functions. The exocrine acini secrete digestive enzymes (amylase, lipase, and protease) into the duodenum through ducts, and the endocrine cells in the islets produce peptide hormones, which maintain a balance between anabolism and catabolism. Endocrine beta-cell secrete insulin and amylin; alpha-cells secrete glucagon; delta-cells secrete somatostatin; PP-cells secrete pancreatic polypeptide; and epsilon-cells secrete ghrelin [8].

Type 1 diabetes mellitus (T1DM) is characterized by absolute insulin deficiency induced by the autoimmune destruction of pancreatic beta-cells. Type 2 diabetes mellitus (T2DM) is characterized by relative insulin deficiency, in which insulin secretion is insufficient to overcome insulin resistance. Beta-cell function is estimated to decrease by about 50% at the onset of T2DM [9]. Recent genome-wide association studies have identified nearly 75 susceptibility loci associated with T2DM, most of which are thought to be associated with beta-cell failure [10, 11]. Thus, deficits in functional beta-cell mass are commonly recognized in both types of diabetes (~99% deficit in long-standing T1DM, 40-60% deficit in long-standing T2DM) [12-15].

Insulin therapy is the only treatment for T1DM; however, the restoration of physiological insulin secretion by insulin injection is difficult to achieve, and results in unstable glucose levels. Better glycemic control has to be related to more frequent hypoglycemia in both T1DM and T2DM [16-18]. Most adults with T1DM in the USA have been HbA1c level higher than 7.5%, and the mean HbA1c level in Japanese diabetic patients is also high (T1DM, 8.2%; T2DM, 7.4%) [19]. Therefore, the restoration of a functional beta-cell mass is the logical and most effective treatment for diabetes mellitus.

Pancreas transplantation is currently the only known therapy for T1DM that reliably establishes a long-term euglycemic state [20]. It is effective in that patients remain insulin independent for more than 10 years [21]. After transplantation, the normal glucagon response to hypoglycemia is restored, and hypoglycemic episodes are uncommon [22]. However, transplantation requires major surgery and has a surgical complication (repeat laparotomy) rate between 10% and 20% [23].

Pancreatic islet transplantation is safe and reproducible. In this procedure, isolated islets are embolized into the liver through a catheter placed into the main portal vein of the recipient. However, insulin independence rarely extends beyond two years [24-27]. The necessary immunosuppressive regimen, including a calcineurin inhibitor or mTOR inhibitor, and the process of islet isolation itself may contribute to the low islet viability [28-30]. Both pancreas and islet transplantation require immunosuppressive therapy, carry the threat of recurrence of autoimmunity, and are limited by donor shortages [31-35].

Patient-derived induced pluripotent stem (iPS) cells may provide an unlimited supply of transplantable cells for beta-cell replacement therapy in diabetic patients. Autotransplantation avoids the risks associated with allograft rejection and the need for immunosuppressants. Previous studies have demonstrated clearly that the most efficient and reproducible method to generate a given cell type from stem cells is to recapitulate embryonic development *in vitro* [36, 37]. This would be the case for both the endocrine pancreas (Fig. 1) and adipocytes [38, 39]. Further understanding of the developmental processes enables us to design more robust and reliable differentiation protocols for the treatment of diabetes mellitus.

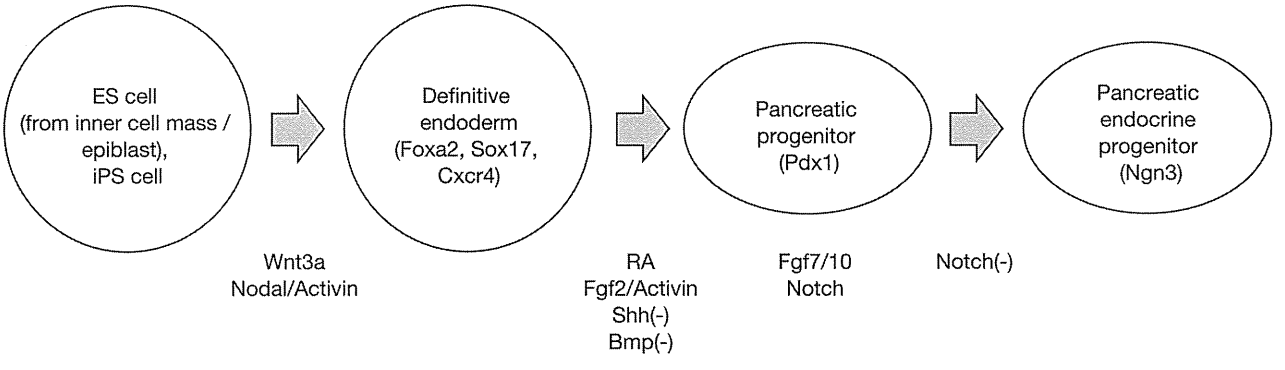

Fig. 1 Simplified representation of the signaling pathways regulating pancreatic development

Endocrine pancreatic development

Endodermal origin of the pancreas

The origin of pancreatic endocrine cells from the neuroectoderm (neural crest) was proposed around 1970 [40]. Pancreatic endocrine cells and neurons share common biochemical properties, morphological features, and molecules; for example, neuron-specific enolase, synaptophysin, chromogranin A, Pax4, Pax6, NeuroD, Nkx2.2, Nkx6.1, Isl1, MafB, and MafA [41, 42]. However, fate-mapping studies using quail-chick chimeras provided evidence against a neuroectoderm origin for pancreatic endocrine cells [43-45]. Furthermore, *ex vivo* organ culture experiments demonstrated that a rat embryo without the neurectoderm can form a normal pancreas, indicating that all types of pancreatic cells are derived from endoderm progenitors [46].

Definitive endoderm (DE) formation

The endoderm is divided into two types: the visceral endoderm (VE), which derives directly from the inner cell mass; and the other is the DE [47]. The VE forms the yolk sac but rarely contributes to the embryo proper [48-51]. The DE forms during gastrulation, when DE progenitors ingress into the anterior primitive streak and migrate into and replace the VE layer. The DE and VE share common transcription factors such as Foxa2 and Sox17, but only the DE expresses chemokine receptor (Cxcr)-4 [52]. Wnt/beta-catenin signaling is detected in the primitive streak [53]. Wnt3- or beta-catenin-knockout mice lack a primitive streak [54, 55]. Nodal, a TGF-beta family member, is expressed in the anterior primitive streak and is required for the specification of the anterior DE [56, 57]. Induction of DE differentiation from embryonic stem (ES)/iPS cells is accomplished by adding TGF-beta ligands (Nodal, Activin A) and either Wnt molecules (Wnt3a) or a GSK3-beta inhibitor [52, 58-63].

Pancreatic specification

During organogenesis, the DE forms the epithelial lining of the primitive gut tube from which the digestive tract, thyroid, liver, and pancreas develop [64]. The pancreas develops from the ventral and dorsal buds of the endoderm expressing the pancreatic-duodenal homeobox gene (Pdx1) [65]. In the Pdx1-knockout mouse, the pancreas fails to develop beyond the bud stage [66, 67].

The retinoic acid (RA)-synthesizing enzyme, retinaldehyde dehydrogenase 2 (Raldh2), is expressed in the somitic mesoderm dorsal to the primitive gut tube. Raldh2-knockout mice lack expression of Pdx1 in the dorsal endoderm, but administration of RA rescues the loss of Pdx1 expression [68, 69]. Treatment of ES cell-derived endoderm cells with RA induces the expression of Pdx1 as well as other important transcription factors, such as pancreatic transcription factor-1a and neurogenin 3 (Ngn3) [70, 71].

The Hedgehog (Hh) family of proteins controls cell growth, survival, and fate, and patterns almost every aspect of the vertebrate body plan [72]. The Hh family of proteins also plays a role in the maintenance of many adult structures that include proliferating cell populations [73]. Sonic hedgehog (Shh) is expressed throughout the embryonic gut tube, except for the pancreatic bud endoderm [74, 75]. Forced expression of Shh from the Pdx1 promoter inhibits pancreatic development [74]. The Shh inhibitor cyclopamine expands the endodermal region where pdx1 expression starts [76]. Thus, the absence of Shh provides a permissive condition leading to pancreatic specification. Reciprocal antagonism between Hh and RA signaling has been suggested [77].

In addition to RA, several other mesodermal signals are critical for patterning the embryonic endoderm into the pancreas. The notochord, the future backbone, is located proximal to the dorsal prepancreatic endoderm. Notochord-derived signals (fibroblast growth factor 2 (Fgf2) and Activin) can suppress endodermal Shh expression and initiate pancreatic differentiation [75, 78, 79]. After notochordal contact with the prepancreatic endoderm, endothelial signals from the nearby aorta promote further dorsal pancreatic specification [80, 81]. The lateral plate mesoderm that lies beneath the ventral prepancreatic endoderm also sends instructive signals that establish the ventral pancreatic domain [82].

There is a bipotential precursor population for the pancreas and liver within the embryonic endoderm [83]. Bone morphogenetic protein (BMP) from cardiogenic mesoderm adjacent to the prehepatic endoderm induces hepatic genes and excludes the pancreatic fate [84, 85]. BMP inhibition by Noggin or other inhibitors at this step is the common basis for the *in vitro* pancreatic differentiation protocols from ES/iPS cells [71, 86-91].

Pancreatic epithelial cell growth and endocrine commitment

The Fgf7 subfamily is unique among FGFs because its members (Fgf7, Fgf10, and Fgf22) are expressed exclusively in the mesenchyme and interact specifically with the Fgf receptor 2b [92]. Fgf7 and 10 are expressed in the mesenchyme adjacent to the prepancreatic buds, and Fgf receptor 2b is expressed in the pancreatic epithelium; addition of each fgf to organ cultures promotes the proliferation of pancreatic epithelial cells [93-95]. In the Fgf10-knockout mouse, pancreatic hypoplasia and the absence of islet cells are evident [93]. Conversely, transgenic overexpression of Fgf10 in Pdx1-expressing progenitor cells increases the proliferation of epithelial cells and blocks differentiation by activating Notch signaling [95, 96].

Notch signaling is known to be responsible for the maintenance of neuronal stem cell populations by inhibiting their differentiation [97]. Targeting of Notch pathway genes in mice results in the upregulation of Ngn3 and premature endocrine differentiation at the expense of progenitor cell proliferation, suggesting that activation of the pathway plays a role in maintaining the progenitor cell state in the early pancreatic epithelium [98-100]. Ngn3 is a basic helix-loop-helix transcription factor that regulates the development of hypothalamic neurons [101]. In the Ngn3-knockout mouse, all pancreatic endocrine cell lineages and endocrine cell-related transcription factors (such as Isl1, Pax4, Pax6, and NeuroD) are lost [102].

Conditional knockout mice lacking Smad4 (a common transcriptional coactivator in the pathway) in Pdx1-expressing pancreatic progenitors can generate a normal pancreas, suggesting that TGF-beta/BMP signaling is dispensable after pancreatic commitment [103]. Inhibition of TGF-beta and BMP signaling has been reported to increase commitment to endocrine lineage [89].

Pancreatic beta-cell differentiation and maturation

A complex cascade of many transcription factors, such as Isl1, Pax4, Pax6, NeuroD, Nkx2.2, Nkx6.1, Isl1, MafB, and MafA is involved in the differentiation and maturation of pancreatic beta-cells, but little is known about the extrinsic signals regulating this process [104]. Many differentiation protocols for inducing insulin-producing cells from human ES/iPS cells have been reported [71, 87-90, 105-110]. However, most insulin-producing cells generated are immature, produce multiple hormones and low levels of insulin, and have poor responses to secretory stimuli. In addition, all types of pancreatic cells, not just beta-cells, are differentiated at the same time in most protocols. Further studies are now trying to obtain more fully differentiated beta-cells in the presence of specific cues.

Adipocyte transplantation

In mammals, adipose tissue comprises white adipose tissue (WAT), primary site of energy storage and mobilization in the form of triglyceride and brown adipose tissue (BAT), which specializes in energy dissipation as thermogenesis.

Obesity is an excess adiposity and is linked to T2DM, cardiovascular, pulmonary, liver, and kidney diseases and certain types of cancer [111, 112]. The anatomical distribution of WAT influences the risks associated with obesity. Obese individuals with a high waist-to-hip ratio, indicating increased visceral fat, have a higher risk for metabolic abnormalities than do individuals with a low waist-to-hip ratio [113]. Subcutaneous fat differs intrinsically from visceral fat and is thought to protect from metabolic disorders [114].

Lipodystrophy is an abnormal loss of adiposity and is characterized by loss of body fat and insulin resistance [115]. Lipodystrophy is accompanied by diabetes mellitus, hypertriglyceridemia, and hepatic steatosis. Leptin treatment or transplantation of wild-type but not leptin-deficient WAT rescued the phenotype of A-ZIP/F-1 lipodystrophic mice, suggesting that leptin deficiency is the major contributor to the metabolic complications of lipodystrophy [116-119]. Several studies have shown that intraperitoneal transplantation of WAT improves glucose tolerance and insulin sensitivity in mice with other conditions besides lipodystrophy [114, 120].

BAT is a major site of energy dissipation because of its high mitochondrial content; in BAT mitochondria, oxidative phosphorylation is uncoupled from adenosine triphosphate (ATP) production as a result of proton leak catalyzed by uncoupling protein 1 (UCP1) [121]. Studies of mice lacking BAT or UCP1 have demonstrated the ability of BAT thermogenesis to protect against diet induced obesity [122, 123]. Transplantation of BAT into the visceral cavity in mice improved glucose tolerance, increased insulin sensitivity, reduced body weight and fat mass, and reversed high-fat diet-induced insulin resistance [124]. Recent studies have detected metabolically active BAT in the neck and upper thorax of normal humans [125].

Therapeutic adipogenesis is an exciting frontier of

metabolic medicine, but our understanding of adipocyte development is still poor compared with that of pancreatic development [38, 39].

Adipocyte development

White adipocytes

White adipocytes can be generated from both mesoderm and neuroectoderm through mesenchymal stem cells (MSCs) [126]. MSCs are defined by plastic-adherent growth and the potential to give rise to multiple mesenchymal cell lineages including osteocytes, chondrocytes, and adipocytes [127, 128]. MSCs reside in the vascular stroma of adipose tissue, the bone marrow and many other tissues [129, 130]. Several factors that commit or inhibit the conversion of MSCs to the adipocyte lineage have been identified. BMP-2 and -4 signaling supports white adipocyte differentiation [131-134]. Wnt signaling acts as an activator of lineage commitment from MSCs to white adipocytes and later as an inhibitor of the differentiation program [135-138]. Shh signaling has an inhibitory effect on adipocyte differentiation [139]. About 10% of adipocytes are renewed annually at all adult ages in humans [140].

Brown adipocytes

BAT originates from the paraxial mesoderm [141]. Brown adipocytes and skeletal muscle develop from a common progenitor, which expresses Myf5 [142]. BMP-7 triggers commitment of progenitor cells to a brown adipocyte lineage by inducing the regulator PRD1-BF1-RIZ1 homologous domain containing 16 (PRDM16) and peroxisome proliferator-activated receptor (PPAR) -gamma-coactivator-1 alpha [143]. PRDM16 specifies the brown adipocyte lineage from the Myf5-expressing progenitors by activating the transcriptional function of PPAR-gamma and by suppressing myogenic factors [144, 145].

Patient-derived iPS cells for mitochondrial disease modeling

The ability to generate iPS cells from patients allows one to obtain genetically identical cells of clinical interest for pathogenesis modeling. Patient-specific iPS cell lines have been derived from individuals with several endocrine and metabolic diseases (Table 1). In some cases, *in vitro* differentiation of iPS cells to the affected cell types has been reported, and some of which successfully recapitulate disease-associated abnormalities. A deeper understanding in cell differentiation and function will be needed for iPS cell-based disease modeling.

We recently generated iPS cells from patients with the A3243G mitochondrial DNA (mtDNA) mutation (Mt-iPS cells) [146]. mtDNA is present inside mitochondria and codes for enzymes for ATP production [147]. In mtDNA disease, wild-type and mutant mtDNA coexist in the same cell in a state called heteroplasmy. The tRNA (*Leu*) A3243G mutation is observed frequently in mtDNA diseases and is associated with diabetes mellitus, hearing loss, and cardiomyopathy [148]. The mode of inheritance of mtDNA diseases is maternal because sperm-derived paternal mtDNA disappears during early embryogenesis, [149]. However, the penetrance of mtDNA disease is variable, and it is not possible to predict the phenotypes of a child from the mother's heteroplasmy level [150]. This is also the case for somatic cells: it is not possible to predict to which cell types the mutant mtDNA will dominantly migrate during development.

A striking feature of these Mt-iPS cells was their bimodal levels of heteroplasmy (Fig. 2). The mtDNA mutation frequencies decreased to undetectable levels in about half of the clones, whereas the levels of mutation heteroplasmy were higher in the other half of the clones compared with those in the patients' original fibroblasts. The mtDNA content did not differ significantly between mutation-free and mutation-rich Mt-iPS cells.

To date, there is no specific therapy or cure for mitochondrial diseases. Efforts to understand the mtDNA diseases have been hampered by the lack of a disease model. Mutation-rich Mt-iPS cells may provide a suitable source of cells for human mitochondrial disease modeling *in vitro*. In addition, mutation-free iPS cells could provide an unlimited supply of disease-free cells for autologous transplantation therapy.

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Disclosure

No potential conflicts of interest were disclosed.

Table 1 Endocrine-metabolic disease-specific iPS cells based on published literature

Disease	Publication	Genetic cause	Age/Gender	Differentiation into affected cell types: Functional Analysis
Type 1 diabetes	[151]	Multifactorial	42y/Female	ND
	[152]	Multifactorial	30y/Male, 32y/Male	Pancreatic endocrine cell
Type 2 diabetes	[153]	Multifactorial	68y/Female, 78y/Male	Insulin-producing cell
Mitochondrial diabetes	[146]	Mitochondrial genome A3243G	38y/Male, 46y/Female	ND
Prader-Willi syndrome	[154]	Paternal deletion of chromosome 15q11-q13.	ND	ND
Lesch-Nyhan syndrome	[151]	Hypoxanthine phosphoribosyltransferase 1 gene, heterozygous carrier	34y/Female	ND
Familial hypercholesterolemia	[155]	Autosomal dominant mutation in low density lipoprotein receptor	Unknown	Hepatocyte: Impaired ability to incorporate low density lipoprotein
	[156]	Low density lipoprotein receptor gene: 5 kb deletion (FG381)	14y/Male	
X-linked adrenoleukodystrophy	[157]	ATP-binding-cassette transporter superfamily D member 1 gene: deletion from exon 8 to exon 10, homozygous	6y/Male	Oligodendrocyte: Excessive very long chain fatty acids accumulation
		ATP-binding-cassette transporter superfamily D member 1 gene: deletion from exon 7 to exon 10, homozygous	32y/Male	
Glycogen storage disease type 1	[158]	Glucose-6-phosphate transporter gene: c.1124-2A>G	7y/Male	Hepatocyte
	[155]	ND	25y/Male	Hepatocyte: Excessive glycogen and lipid accumulation and excessive production of lactate
Gaucher disease	[151]	Glucocerebrosidase gene: pAsn370Ser, c.84-85insG , compound heterozygous	20y/Male	ND
	[159]	Glucocerebrosidase gene: pAsn370Ser, c.84-85insG , compound heterozygous	20y/Male	Neuron
	[160]	Glucocerebrosidase gene: pAsn370Ser, homozygous	Adult/ND	Monocyte/Macrophage, Neuron: Reduced glucocerebrosidase activity and accumulation of glucosylsphingolipids
		Glucocerebrosidase gene: p.Leu444Pro, RecNcil, compound heterozygous	Infant/ND	
		Glucocerebrosidase gene: p.Leu444Pro, homozygous	3y/ND	
Wilson's disease	[161]	ATPase, Cu^{2+} transporting, beta polypeptide gene: p.Arg778Leu, homozygous	Middle age/Male	Hepatocyte: Defective copper transport
Crigler-Najjar syndrome	[158]	Uridine diphosphate glucuronosyltransferase 1A1 gene: p.Leu413Pro, homozygous	19y/Female, 21y/Male	Hepatocyte
	[155]	Uridine diphosphate glucuronosyltransferase 1A1 gene: 13 bp deletion in exon2, homozygous	2m/Male	ND
Alpha 1-antitrypsin deficiency	[155]	Alpha 1-antitrypsin gene: p.Glu342Lys, homozygous	16y/Male, 55y/Male, 65y/Male	Hepatocyte: Retention of misfolded polymeric alpha 1-antitrypsin protein within the endoplasmic reticulum
	[162]	ND	4m/Male, 47y/Female, 57y/Female, 61y/Female, 64y/Female	Hepatocyte
Tyrosinemia type 1	[158]	Fumarylacetoacetate hydrolase gene: p.Gln64His, homozygous	6y/Female	Hepatocyte
	[155]	Fumarylacetoacetate hydrolase gene: p.Val166Gly, heterozygous	2m/Male	ND
Progressive familial hereditary Cholestasis	[158]	Multifactorial	17y/Female	Hepatocyte

ND, not described

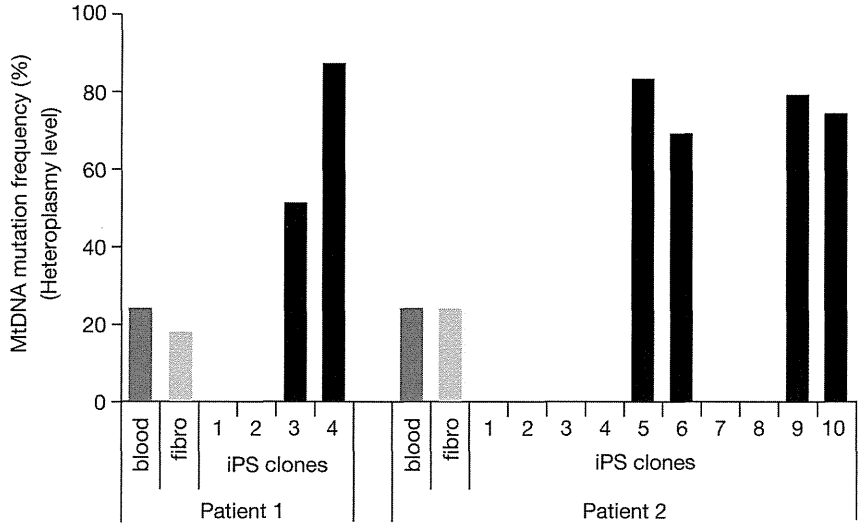

Fig. 2 mtDNA mutation frequencies in Mt-iPS cells
A3243G mtDNA mutation frequencies in the blood cells, original fibroblasts, and Mt-iPS clones from two diabetic patients with the A3243G mtDNA mutation.

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ORIGINAL

NUDT3 rs206936 is associated with body mass index in obese Japanese women

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Abstract. The predominant risk factor of metabolic syndrome is intra-abdominal fat accumulation, which is determined by waist circumference, waist-hip ratio measurements and visceral fat area (VFA); the latter can be accurately measured by performing computed tomography (CT). In addition to environmental factors, genetic factors play an important role in obesity and fat distribution. New genetic loci associated with body mass index (BMI) and adiposity have been identified by genome-wide association studies (GWASs). This study utilized CT to investigate whether single nucleotide polymorphisms (SNPs) that confer susceptibility to higher BMI are associated with VFA, subcutaneous fat area (SFA), and the ratio of VFA to SFA (V/S ratio). We measured the VFA and SFA of 1424 obese Japanese subjects (BMI $\geq$ 25 kg/m^2, 635 men and 789 women) who were genotyped for 13 single nucleotide polymorphisms (SNPs) reported by recent GWASs, namely, *TNNI3K* rs1514175, *PTBP2* rs1555543, *ADCY3* rs713586, *IRS1* rs2943650, *POC5* rs2112347, *NUDT3* rs206936, *LINGO2* rs10968576, *STK33* rs4929949, *MTIF3* rs4771122, *SPRY2* rs534870, *MAP2K5* rs2241423, *QPCTL* rs2287019, and *ZC3H4* rs3810291. The G-allele of *NUDT3* rs206936 was significantly associated with increased BMI ($P = 5.3 \times 10^{-5}$) and SFA ($P = 0.00039$) in the obese Japanese women. After adjustment with BMI, the association between rs206936 and SFA was not observed. This significant association was not observed in the men. The other SNPs analyzed were not significantly associated with BMI, VFA, SFA, or V/S ratio. Our results suggest that *NUDT3* rs206936 is associated with BMI in Japanese women.

Key words: *NUDT3*, Visceral adipose tissue, Subcutaneous fat tissue, Computed tomography, Japanese

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RECENTLY, the change in dietary habits and lifestyle in Asian countries including Japan have led to excessive and abundant nutrition and subsequent increase in obesity levels, which is a key feature of metabolic syn-

drome. Specifically, abnormal fat distribution is more closely related to metabolic syndrome than increased absolute fat volume [1-2]. The accumulation of intra-abdominal visceral fat plays a major role in metabolic syndrome, as the accumulated visceral adipose tissue leads to alterations in the plasma levels of adipocytokines, which thereby result in the development of dyslipidemia, hypertension, and insulin resistance [2, 3]. Numerous evidences support the fact that body fat distribution is influenced by genetic loci [4-7]. Fat distribution is determined on the basis of waist circumference, waist-hip ratio, biological impedance, or visceral fat area (VFA) measured using computed tomography (CT) [1, 8, 9]. Recent progress using genome-wide association studies (GWASs) has identified 17 loci linked to waist circumference or waist-hip ratio [10-12]. Very recently, 3 genetic loci associated with VFA and subcutaneous fat area (SFA) determined using CT were identified by GWASs [13]. We have examined the reported loci, and determined that single nucleotide polymorphisms (SNPs) in the fat mass- and obesity-associated (*FTO*) gene were significantly linked to SFA and that lysophospholipase-like protein 1 (*LYPLAL1*) rs4846567 is associated with the ratio of VFA to SFA (V/S ratio) in the Japanese population [14-16].

Recent progress in GWASs has increased the number of known genetic susceptibility loci for obesity [17-19]. We have reported that among SNPs that increase susceptibility to obesity, rs7498665 in the SH2B adaptor protein 1 (*SH2B1*) gene is specifically associated with VFA [20]. Recent report has identified 18 novel loci that influence susceptibility to obesity [21], and genetic variation in the insulin receptor substrate 1 (*IRS1*) and sprouty homolog 2 (*SPRY2*) genes have been associated with adiposity [22]. Among 20 SNPs, 6 SNPs are monomorphic in the Japanese population: rs2890652 in the low density lipoprotein receptor-related protein 1B (*LRP1B*) gene; rs887912 in the *FLJ30838* gene; rs13078807 in the cell adhesion molecule 2 (*CADM2*) gene; rs13107325 in the solute carrier family 39 (zinc transporter), member 8 (*SLC39A8*) gene; rs11847697 in the protein kinase D1 (*PRKD1*) gene; and rs12444979 in the G protein-coupled receptor, family C, group 5, member B (*GPRC5B*) gene (from HapMap database). rs4836133 in the zinc finger protein 608 (*ZNF608*) gene is tri-allelic in the Japanese population.

It is not clear whether novel body mass index (BMI) or adiposity-associated SNPs affect the accumulation of visceral or subcutaneous fat mass in obese Japanese. Thus, we investigated whether the recently reported BMI or adiposity associated SNPs also affect VFA, SFA, and V/S ratio in the obese Japanese.

Materials & Methods

Subjects

We enlisted 1424 Japanese subjects who had visited 9 outpatient clinics during 2002 to 2011, to undergo treatment for obesity (BMI $\geq$ 25 kg/m^2) with or without metabolic abnormalities such as hypertension, dyslipidemia, and type 2 diabetes. Obesity (BMI $\geq$25 kg/m^2) was diagnosed according to the Japanese obesity criteria [23]. Patients with secondary obesity and obesity-related hereditary disorders were excluded from this study. The patients agreed to undergo CT testing (in the supine position) to determine the VFA, and SFA values at the umbilical level (L4–L5), as previously reported [24]. The values of VFA, and SFA were calculated using the FatScan software program (N2system, Osaka, Japan) [24]. Clinical data were recorded at the first visit to the hospital, and the clinical characteristics of the subjects are summarized in Table 1. Written informed consent was obtained from each subject, and the protocol was approved by the ethics committee of each institution and Kyoto University.

DNA extraction and SNP genotyping

Genomic DNA was extracted from the blood samples collected from each subject using the Genomix kit (Talent Srl, Trieste, Italy). We selected 13 SNPs that were recently identified as loci associated with BMI or adiposity by meta-analysis [21, 22], and constructed Invader probes (Third Wave Technologies, Madison, WI, USA). The following 13 SNPs were genotyped and used for analysis: rs1514175 in the TNNI3 interacting kinase (*TNNI3K*) gene; rs1555543 in the polypyrimidine tract binding protein 2 (*PTBP2*) gene; rs713586 in the adenylate cyclase 3 (*ADCY3*) gene; rs2943650 in the *IRS1* gene; rs2112347 in the POC5 centriolar protein homolog (*POC5*) gene; rs206936 in the nudix (nucleoside diphosphate linked moiety X)-type motif 3 (*NUDT3*) gene; rs10968576 in the leucine rich repeat and Ig domain containing 2 (*LINGO2*) gene; rs4929949 in the serine/threonine kinase 33 (*STK33*) gene; rs4771122 in the mitochondrial translational initiation factor 3 (*MTIF3*) gene; rs534870 in the *SPRY2* gene; rs2241423 in the mitogen-activated protein

Table 1 Clinical characteristics of the subjects

	Men	Women	Total
n	635	789	1424
Age (years)	48.6 ± 12.5	52.3 ± 11.3	50.7 ± 12.0
BMI (kg/m^2)	29.9 ± 6.0	28.2 ± 5.2	29.0 ± 5.6
VFA (cm^2)	153.9 ± 66.6	102.6 ± 54.1	125.5 ± 65.1
SFA (cm^2)	205.5 ± 108.3	243.3 ± 97.7	226.5 ± 104.3
Fasting plasma glucose (mg/dL)	109.4 ± 31.9	108.4 ± 36.5	108.9 ± 34.5
Fasting insulin (μU/mL)[a]	13.6 ± 17.9	10.4 ± 10.3	11.8 ± 14.3
HOMA-IR[a]	3.9 ± 7.5	2.9 ± 3.8	3.4 ± 5.8
Total cholesterol (mg/dL)	211.4 ± 37.1	220.1 ± 38.6	216.2 ± 38.2
Triglycerides (mg/dL)	170.0 ± 147.9	120.6 ± 80.8	142.6 ± 118.1
HDL-cholesterol (mg/dL)	51.7 ± 13.9	61.0 ± 16.0	56.8 ± 15.8
Systolic blood pressure (mmHg)	131.4 ± 16.8	130.5 ± 18.4	130.9 ± 17.8
Diastolic blood pressure (mmHg)	84.7 ± 12.5	80.7 ± 11.2	82.4 ± 12.0

HOMA-IR was assessed as fasting insulin (μU/mL) × fasting plasma glucose (mg/dL) /405. Data are represented as means ± SD. [a], n = 585 in men and n = 727 in women.

kinase kinase 5 (*MAP2K5*) gene; rs2287019 in the glutaminyl-peptide cyclotransferase-like (*QPCTL*) gene; and rs3810291 in the zinc finger CCCH-type containing 4 (*ZC3H4*) gene. The SNPs were genotyped using Invader assays, as previously described [25]. The success rate of these assays was >99.0%.

Statistical analysis

For the additive model, we characterized the genotypes as 0, 1, or 2 depending on the number of copies of the risk alleles. For the dominant model, homozygosity and heterozygosity with the risk allele were coded as 1 and the other was coded as 0. For the recessive model, homozygosity with the risk allele was coded as 1 and others were coded as 0. Risk alleles refer to the BMI-associated alleles previously identified [21, 22]. Multiple linear regression analyses were performed to test the independent effect per allele of each SNP on BMI, VFA, SFA, and V/S ratio, by accounting for the effects of the other variables (i.e., age, sex, and BMI). The BMI, VFA, SFA, and V/S ratio values were logarithmically transformed before performing the multiple linear regression analysis. Hardy–Weinberg equilibrium was assessed using the χ^2-test [26]. The statistical analysis was performed using R software (http://www.r-project.org/). *P*-values were assessed by using Bonferroni correction, and a *P*-value of <0.00096 (0.05/13 SNPs/4 traits) was considered statistically significant.

Results

The clinical characteristics and genotypes of the subjects are shown in Tables 1 and Supplementary Table 2, respectively. All the SNPs were in Hardy–Weinberg equilibrium, except rs4929949 ($P = 0.040$) and rs3810291 ($P = 0.026$) in men, and rs2943650 in women ($P = 0.040$). The risk allele frequencies did not diverge from those reported in the HapMap database. We confirmed that 6 SNPs were monomorphic in the Japanese population: rs2890652 in the *LRP1B* gene, rs887912 in the *FLJ30838* gene, rs13078807 in the *CADM2* gene, rs13107325 in the *SLC39A8* gene, rs11847697 in the *PRKD1* gene, and rs12444979 in the *GPRC5B* gene. We found that rs4836133 in the *ZNF608* gene was tri-allelic in the population (A, 43.9%; C, 33.4%; G, 22.7%).

The BMI, VFA, and SFA are known to be affected by gender [27], and therefore, we independently compared BMI-associated SNPs with the fat distribution parameters (BMI, VFA, and SFA) in men and women. Multiple linear regression analyses of the anthropometric parameters with respect to the 13 analyzed SNPs are shown in Tables 2, 3, 4, and 5. The G-allele of rs206936 was significantly associated with BMI in women ($P = 5.3 \times 10^{-5}$) but not in men ($P = 0.19$). SNP rs1555543 in the *PTBP2* gene was weakly associated with BMI in men ($P = 0.017$, Table 2): however, its association was not significant. Two SNPs, namely, rs2112347 in the *POC5* gene ($P = 0.032$) and rs206936 in the

Table 2 Association of the 13 SNPs with BMI in men and women

SNP ID	Nearby gene	Men					Women				
		Genotypic means (kg/m^2)			Effect per risk allele		Genotypic means (kg/m^2)			Effect per risk allele	
		11	12	22	β (s.e.)	*P*-value	11	12	22	β (s.e.)	*P*-value
rs1514175	*TNNI3K*	29.5 ± 5.3	30.9 ± 7.3	31.0 ± 4.9	-0.014 (0.006)	0.015	28.1 ± 5.0	28.3 ± 5.7	28.7 ± 4.8	-0.003 (0.005)	0.59
rs1555543	*PTBP2*	27.7 ± 4.2	29.4 ± 4.5	30.2 ± 6.4	0.014 (0.006)	**0.017**	31.2 ± 6.7	28.4 ± 5.8	28.0 ± 4.9	-0.009 (0.005)	0.095
rs713586	*ADCY3*	30.0 ± 6.0	30.1 ± 6.4	29.4 ± 4.9	0.001 (0.004)	0.77	28.1 ± 5.0	28.2 ± 5.2	28.4 ± 5.6	-0.002 (0.004)	0.66
rs2943650	*IRS1*	31.6 ± 5.3	30.1 ± 5.1	29.9 ± 6.2	-0.008 (0.007)	0.25	26.8 ± 3.9	28.4 ± 6.0	28.2 ± 5.1	0.002 (0.006)	0.78
rs2112347	*POC5*	30.1 ± 6.0	30.0 ± 5.8	29.2 ± 4.8	-0.005 (0.004)	0.24	28.1 ± 5.8	28.3 ± 4.8	28.3 ± 5.2	0.004 (0.004)	0.28
rs206936	*NUDT3*	30.2 ± 6.1	30.2 ± 6.3	29.4 ± 5.5	-0.006 (0.004)	0.19	27.1 ± 4.7	28.0 ± 5.1	29.0 ± 5.6	0.015 (0.004)	**5.3×10^{-5}**
rs10968576	*LINGO2*	30.1 ± 6.2	29.6 ± 5.7	30.0 ± 4.3	-0.001 (0.005)	0.79	28.2 ± 5.3	28.2 ± 5.0	27.5 ± 5.3	-0.003 (0.005)	0.60
rs4929949	*STK33*	29.8 ± 4.9	29.9 ± 6.2	30.0 ± 6.2	0.001 (0.004)	0.76	28.3 ± 5.4	28.5 ± 5.3	27.7 ± 4.9	0.005 (0.004)	0.14
rs4771122	*MTIF3*	29.8 ± 5.8	30.2 ± 6.4	30.4 ± 4.5	0.007 (0.005)	0.16	28.2 ± 5.1	28.1 ± 5.1	29.1 ± 7.3	0.002 (0.004)	0.69
rs534870	*SPRY2*	29.8 ± 6.2	30.0 ± 6.1	29.9 ± 5.4	-0.002 (0.004)	0.62	28.0 ± 5.0	28.3 ± 5.3	28.4 ± 5.3	-0.003 (0.004)	0.51
rs2241423	*MAP2K5*	29.7 ± 5.6	29.9 ± 6.1	30.7 ± 6.6	0.005 (0.004)	0.24	27.7 ± 5.0	28.6 ± 5.2	28.4 ± 5.8	0.006 (0.004)	0.13
rs2287019	*QPCTL*	30.0 ± 6.2	29.7 ± 5.5	30.1 ± 4.8	0.001 (0.005)	0.92	28.1 ± 5.1	28.3 ± 5.3	27.4 ± 4.5	-0.001 (0.005)	0.82
rs3810291	*ZC3H4*	31.1 ± 7.4	30.1 ± 6.2	29.6 ± 5.6	0.007 (0.005)	0.13	26.9 ± 3.4	28.3 ± 5.4	28.2 ± 5.2	-0.004 (0.004)	0.32

Data are expressed as means ± SD. Allele1, allele 2, and the risk allele of each SNP are indicated in Supplementary Table 1. The effect size and *P*-values are derived from a linear regression analysis. BMI was adjusted for age, and log-transformed for the analysis. Number in bold indicates $P < 0.05$. 11, allele1/allele1; 12, allele1/allele2; 22, allele2/allele2.

Table 3 Association of the 13 SNPs with VFA in men and women

SNP ID	Nearby gene	Men						Women					
		Genotypic means (cm^2)			Effect per risk allele			Genotypic means (cm^2)			Effect per risk allele		
		11	12	22	β (s.e.)	*P*-value	*P*-value*	11	12	22	β (s.e.)	*P*-value	*P*-value*
rs1514175	*TNNI3K*	152.6 ± 68.9	157.1 ± 61.8	157.5 ± 59.7	-0.024 (0.016)	0.14	0.74	103.3 ± 53.7	101.8 ± 56.1	93.3 ± 44.2	0.013 (0.018)	0.45	0.16
rs1555543	*PTBP2*	130.9 ± 70.1	150.4 ± 66.4	155.8 ± 66.3	0.027 (0.016)	0.098	0.58	129.7 ± 60.9	104.9 ± 54.7	101.3 ± 53.6	-0.020 (0.018)	0.29	0.97
rs713586	*ADCY3*	160.6 ± 69.0	151.9 ± 63.2	151.8 ± 71.9	0.020 (0.012)	0.11	0.093	101.7 ± 49.6	98.3 ± 51.3	112.8 ± 63.6	-0.012 (0.013)	0.33	0.36
rs2943650	*IRS1*	148.5 ± 59.3	165.5 ± 75.7	151.2 ± 64.2	-0.029 (0.019)	0.13	0.27	92.9 ± 38.4	110.3 ± 57.0	101.4 ± 53.7	-0.032 (0.022)	0.15	**0.041**
rs2112347	*POC5*	159.6 ± 66.3	151.1 ± 67.0	152.0 ± 65.6	-0.014 (0.012)	0.26	0.52	96.9 ± 58.5	105.3 ± 50.8	104.2 ± 52.5	0.027 (0.013)	**0.032**	0.058
rs206936	*NUDT3*	155.2 ± 65.4	155.3 ± 67.7	151.3 ± 66.0	-0.008 (0.012)	0.53	0.99	99.9 ± 54.7	100.3 ± 55.3	107.6 ± 51.7	0.030 (0.013)	**0.018**	0.88
rs10968576	*LINGO2*	152.4 ± 66.5	157.2 ± 68.5	158.5 ± 58.5	0.014 (0.015)	0.34	0.22	102.4 ± 53.0	103.9 ± 55.8	93.7 ± 58.2	-0.004 (0.016)	0.83	0.89
rs4929949	*STK33*	163.1 ± 72.7	154.1 ± 64.1	149.8 ± 67.8	0.018 (0.013)	0.16	0.16	109.5 ± 58.6	102.4 ± 54.3	98.5 ± 50.1	0.018 (0.013)	0.16	0.50
rs4771122	*MTIF3*	152.9 ± 68.8	155.5 ± 62.8	153.4 ± 69.1	0.008 (0.014)	0.56	0.90	102.9 ± 54.6	101.8 ± 53.7	105.5 ± 51.7	0.004 (0.015)	0.80	0.98
rs534870	*SPRY2*	150.5 ± 60.5	155.1 ± 72.1	157.0 ± 59.8	-0.008 (0.012)	0.53	0.65	103.9 ± 55.6	103.0 ± 54.1	98.9 ± 51.2	0.008 (0.013)	0.55	0.19
rs2241423	*MAP2K5*	158.2 ± 72.5	152.2 ± 62.3	148.9 ± 64.7	-0.007 (0.012)	0.58	0.19	98.5 ± 52.6	105.8 ± 54.7	104.5 ± 55.5	0.024 (0.013)	0.070	0.26
rs2287019	*QPCTL*	153.5 ± 67.9	154.7 ± 65.6	160.1 ± 48.7	-0.013 (0.015)	0.41	0.31	100.8 ± 53.0	105.8 ± 56.0	101.0 ± 52.3	-0.006 (0.016)	0.69	0.74
rs3810291	*ZC3H4*	151.8 ± 65.4	153.7 ± 63.8	153.6 ± 68.2	0.002 (0.013)	0.85	0.52	101.4 ± 47.9	100.0 ± 55.0	104.0 ± 54.0	-0.009 (0.015)	0.53	1.00

Data are expressed as means ± SD. Allele1, allele 2, and the risk allele of each SNP are indicated in Supplementary Table 1. The effect size and *P*-values are derived from a linear regression analysis. Number in bold indicates $P < 0.05$. Log-transformed VFA was adjusted for age. * Log-transformed VFA was adjusted for age, and log-transformed BMI. 11, allele1/allele1; 12, allele1/allele2; 22, allele2/allele2.

Table 4 Association of the 13 SNPs with SFA in men and women

SNP ID	Nearby gene	Men						Women					
		Genotypic means (cm^2)			Effect per risk allele			Genotypic means (cm^2)			Effect per risk allele		
		11	12	22	β (s.e.)	*P*-value	*P*-value*	11	12	22	β (s.e.)	*P*-value	*P*-value*
rs1514175	*TNNI3K*	197.4 ± 102.7	221.5 ± 119.4	223.5 ± 101.3	-0.042 (0.016)	0.0089	0.24	243.1 ± 96.8	242.7 ± 100.1	254.2 ± 102.3	-0.002 (0.012)	0.85	0.80
rs1555543	*PTBP2*	172.0 ± 88.3	198.0 ± 95.3	208.6 ± 112.9	0.030 (0.016)	0.064	0.94	289.7 ± 86.8	247.4 ± 105.0	241.0 ± 94.9	-0.017 (0.012)	0.18	0.89
rs713586	*ADCY3*	209.5 ± 114.9	208.1 ± 108.0	194.4 ± 101.9	0.010 (0.012)	0.41	0.36	239.7 ± 92.4	245.2 ± 100.9	244.4 ± 98.6	-0.003 (0.008)	0.73	0.98
rs2943650	*IRS1*	266.9 ± 140.9	212.2 ± 101.7	202.8 ± 109.1	-0.036 (0.019)	0.052	0.099	227.5 ± 90.9	251.4 ± 100.6	242.1 ± 97.4	-0.010 (0.015)	0.48	0.16
rs2112347	*POC5*	208.6 ± 106.8	206.8 ± 110.8	194.4 ± 100.6	-0.016 (0.012)	0.18	0.49	240.5 ± 105.2	246.3 ± 93.4	242.9 ± 95.7	0.008 (0.008)	0.35	0.87
rs206936	*NUDT3*	216.0 ± 123.0	210.7 ± 106.3	193.1 ± 102.6	-0.019 (0.012)	0.10	0.32	222.9 ± 84.6	243.0 ± 98.1	255.4 ± 102.5	0.031 (0.009)	**0.00039**	0.42
rs10968576	*LINGO2*	208.3 ± 111.7	201.2 ± 103.8	205.0 ± 98.3	-0.003 (0.014)	0.85	0.98	241.5 ± 97.4	247.6 ± 98.0	241.4 ± 102.1	0.005 (0.011)	0.63	0.22
rs4929949	*STK33*	205.4 ± 101.6	206.8 ± 109.7	203.7 ± 110.3	0.009 (0.012)	0.46	0.45	244.3 ± 97.4	246.6 ± 101.1	238.0 ± 92.6	0.008 (0.009)	0.32	0.93
rs4771122	*MTIF3*	201.5 ± 108.8	207.2 ± 103.6	243.5 ± 133.5	0.031 (0.014)	**0.027**	0.075	242.9 ± 98.7	241.1 ± 95.7	264.1 ± 101.9	0.009 (0.010)	0.36	0.37
rs534870	*SPRY2*	208.8 ± 114.6	202.1 ± 109.5	208.7 ± 94.8	-0.009 (0.012)	0.47	0.59	237.9 ± 94.7	244.9 ± 98.6	249.0 ± 101.2	-0.009 (0.009)	0.33	0.53
rs2241423	*MAP2K5*	205.6 ± 106.0	201.8 ± 109.0	217.8 ± 114.0	0.004 (0.012)	0.73	0.40	243.3 ± 98.9	240.5 ± 94.6	253.7 ± 105.1	0.004 (0.009)	0.69	0.32
rs2287019	*QPCTL*	205.3 ± 105.9	202.4 ± 113.1	233.8 ± 115.9	-0.009 (0.015)	0.55	0.30	245.6 ± 100.9	240.2 ± 91.8	225.7 ± 95.1	0.007 (0.011)	0.50	0.20
rs3810291	*ZC3H4*	215.0 ± 123.9	207.1 ± 111.2	202.8 ± 104.2	0.004 (0.013)	0.77	0.19	239.7 ± 82.5	250.3 ± 98.5	240.0 ± 98.8	0.011 (0.010)	0.28	**0.0083**

Data are expressed as means ± SD. Allele1, allele 2, and the risk allele of each SNP are indicated in Supplementary Table 1. The effect size and *P*-values are derived from a linear regression analysis. Log-transformed SFA was adjusted for age. * Log-transformed SFA was adjusted for age, and log-transformed BMI. Number in bold indicates $P < 0.05$. 11, allele1/allele1; 12, allele1/allele2; 22, allele2/allele2.

Table 5 Association of the 13 SNPs with V/S ratio in men and women

SNP ID	Nearby gene	Men					Women				
		Genotypic means (ratio)			Effect per risk allele		Genotypic means (ratio)			Effect per risk allele	
		11	12	22	β (s.e.)	*P*-value	11	12	22	β (s.e.)	*P*-value
rs1514175	*TNNI3K*	0.87 ± 0.40	0.84 ± 0.42	0.76 ± 0.27	0.018 (0.015)	0.24	0.45 ± 0.26	0.44 ± 0.26	0.39 ± 0.17	0.036 (0.035)	0.30
rs1555543	*PTBP2*	0.86 ± 0.46	0.84 ± 0.34	0.86 ± 0.42	-0.003 (0.015)	0.85	0.46 ± 0.15	0.45 ± 0.23	0.45 ± 0.27	-0.007 (0.037)	0.85
rs713586	*ADCY3*	0.90 ± 0.45	0.83 ± 0.37	0.88 ± 0.42	0.010 (0.012)	0.40	0.46 ± 0.25	0.43 ± 0.24	0.48 ± 0.28	-0.022 (0.025)	0.39
rs2943650	*IRS1*	0.65 ± 0.32	0.88 ± 0.45	0.85 ± 0.39	0.007 (0.018)	0.67	0.43 ± 0.19	0.48 ± 0.28	0.45 ± 0.25	-0.049 (0.043)	0.26
rs2112347	*POC5*	0.86 ± 0.36	0.84 ± 0.41	0.90 ± 0.45	0.002 (0.011)	0.84	0.42 ± 0.24	0.47 ± 0.27	0.45 ± 0.23	0.044 (0.025)	0.079
rs206936	*NUDT3*	0.84 ± 0.41	0.84 ± 0.41	0.87 ± 0.40	0.012 (0.011)	0.29	0.47 ± 0.26	0.43 ± 0.25	0.46 ± 0.26	0.000 (0.025)	0.99
rs10968576	*LINGO2*	0.84 ± 0.41	0.88 ± 0.40	0.90 ± 0.37	0.017 (0.014)	0.23	0.46 ± 0.27	0.44 ± 0.23	0.41 ± 0.21	-0.020 (0.033)	0.53
rs4929949	*STK33*	0.87 ± 0.37	0.86 ± 0.42	0.83 ± 0.40	0.008 (0.012)	0.48	0.48 ± 0.28	0.44 ± 0.25	0.44 ± 0.26	0.022 (0.025)	0.38
rs4771122	*MTIF3*	0.86 ± 0.41	0.85 ± 0.40	0.73 ± 0.40	-0.023 (0.013)	0.084	0.45 ± 0.27	0.45 ± 0.24	0.42 ± 0.22	-0.013 (0.030)	0.67
rs534870	*SPRY2*	0.84 ± 0.39	0.87 ± 0.43	0.83 ± 0.35	0.001 (0.011)	0.93	0.47 ± 0.26	0.45 ± 0.26	0.42 ± 0.23	0.038 (0.026)	0.14
rs2241423	*MAP2K5*	0.85 ± 0.38	0.88 ± 0.43	0.78 ± 0.37	-0.011 (0.012)	0.34	0.43 ± 0.25	0.47 ± 0.26	0.43 ± 0.25	0.047 (0.026)	0.073
rs2287019	*QPCTL*	0.84 ± 0.39	0.88 ± 0.44	0.79 ± 0.33	-0.004 (0.014)	0.80	0.44 ± 0.24	0.47 ± 0.28	0.49 ± 0.28	-0.032 (0.032)	0.32
rs3810291	*ZC3H4*	0.81 ± 0.35	0.86 ± 0.39	0.85 ± 0.42	-0.001 (0.012)	0.92	0.45 ± 0.22	0.43 ± 0.28	0.46 ± 0.24	-0.047 (0.030)	0.12

Data are expressed as means ± SD. Allele1, allele 2, and the risk allele of each SNP are indicated in Supplementary Table 1. The effect size and *P*-values are derived from a linear regression analysis. V/S ratio was adjusted for age, and log-transformed for the analysis. 11, allele1/allele1; 12, allele1/allele2; 22, allele2/allele2.

Table 6 Association of the 4 SNPs with BMI, VFA, and SFA in men and women

				Recessive model				Dominant model			
				Adjusted with age		Adjusted with age and BMI		Adjusted with age		Adjusted with age and BMI	
Phenotype	SNP ID	Nearby gene	Sex	β (s.e.)	*P*-value	β (s.e.)	*P*-value	β (s.e.)	*P*-value	β (s.e.)	*P*-value
BMI	rs1555543	*PTBP2*	Men	0.014 (0.007)	**0.036**	-	-	0.034 (0.019)	0.069	-	-
			Women	-0.007 (0.006)	0.23	-	-	-0.046 (0.021)	0.026	-	-
	rs206936	*NUDT3*	Men	-0.007 (0.006)	0.24	-	-	-0.007 (0.008)	0.35	-	-
			Women	0.020 (0.006)	**0.00047**	-	-	0.021 (0.007)	**0.0020**	-	-
VFA	rs2943650	*IRS1*	Men	-0.034 (0.021)	0.099	-0.023 (0.018)	0.21	-0.011 (0.071)	0.88	0.005 (0.062)	0.93
			Women	-0.042 (0.024)	0.086	-0.043 (0.019)	**0.028**	0.018 (0.078)	0.81	-0.020 (0.061)	0.75
	rs2112347	*POC5*	Men	-0.009 (0.022)	0.66	0.003 (0.019)	0.88	-0.024 (0.018)	0.18	-0.017 (0.016)	0.28
			Women	0.012 (0.022)	0.57	0.004 (0.018)	0.80	0.055 (0.019)	**0.0047**	0.042 (0.015)	**0.0065**
	rs206936	*NUDT3*	Men	-0.016 (0.018)	0.38	-0.005 (0.015)	0.73	-0.001 (0.022)	0.96	0.009 (0.019)	0.64
			Women	0.054 (0.019)	**0.0048**	0.013 (0.015)	0.40	0.021 (0.023)	0.38	-0.023 (0.018)	0.20
SFA	rs206936	*NUDT3*	Men	-0.029 (0.017)	0.10	-0.013 (0.011)	0.26	-0.022 (0.022)	0.32	-0.006 (0.014)	0.67
			Women	0.036 (0.013)	**0.0052**	0.003 (0.009)	0.76	0.047 (0.016)	**0.0023**	0.012 (0.011)	0.28
	rs4771122	*MTIF3*	Men	0.076 (0.039)	0.051	0.053 (0.026)	**0.039**	0.030 (0.017)	0.073	0.014 (0.011)	0.22
			Women	0.044 (0.028)	0.11	0.027 (0.019)	0.15	0.005 (0.012)	0.70	0.004 (0.008)	0.66
	rs3810291	*ZC3H4*	Men	0.000 (0.030)	1.00	-0.023 (0.020)	0.24	0.006 (0.017)	0.71	-0.012 (0.011)	0.29
			Women	0.001 (0.026)	0.98	0.032 (0.018)	0.073	0.016 (0.013)	0.19	0.020 (0.009)	**0.017**

Data are expressed as the means ± SD. Allele1, allele 2, and the risk allele of each SNP are indicated in Supplementary Table 1. The effect size and *P*-values are derived from a linear regression analysis. Number in bold indicates $P < 0.05$. 11, allele1/allele1; 12, allele1/allele2; 22, allele2/allele2.

NUDT3 gene ($P = 0.018$), were weakly, but not significantly, associated with VFA in women (Table 3). After adjustment with BMI, no associations were observed. rs2943650 in the *IRS1* gene was associated with reduced VFA, after adjustment of BMI in women ($P = 0.041$), although it is not significant. SNP rs206936 in the *NUDT3* gene was significantly associated with the SFA in women ($P = 0.00039$), but not in men, which is similar to the results obtained with respect to BMI and its association was disappeared after the adjustment of BMI (Table 4). SNP rs4771122 in the *MTIF3* gene was weakly (not significantly), associated with the SFA after adjustment in men ($P = 0.027$). rs3810291 in the *ZC3H4* gene was weakly (not significantly) associated with SFA after adjustment with BMI in women ($P = 0.0083$). No SNP was associated with V/S ratio (Table 5). We analyzed SNPs with *P*-value less than 0.05 in recessive and dominant model. Recessive model fitted better for rs2943650 in the *IRS1* and dominant model for rs2112347 in the *POC5* gene (Table 6). Additive model fitted better for other SNPs.

Obesity, especially visceral fat obesity, is a major risk factor for metabolic disorders [9, 28, 29]. We examined the effect of 12 SNPs on metabolic traits. The G-allele of rs206936 in the *NUDT3* gene, which was significantly associated with BMI and SFA in women, was not associated with any metabolic disorders (Supplementary Table 2), as reported in previous studies [21, 30].

Discussion

We showed that rs206936 was significantly associated with BMI in Japanese women. Similar observation was also reported in Korean population where rs206936 is also associated with BMI [31]. Thus, rs206936 is likely to be associated with BMI in Asian population. However, we were unable to replicate the association of BMI with any other SNPs. We conducted the power analysis of linear regression (additive model) with a significance level of 0.05, using age and gender as explanatory parameters. The estimated effect sizes per allele (regression coefficients) for logarithmically transformed BMI, allele frequency of rs206936 was used. The power of our statistical test was calculated on the basis of these estimated effect sizes and by performing 10,000 simulations. The power for BMI was estimated to be 0.57 in total subjects, 0.26 in men, and 0.98 in women. Therefore, the failure to replicate the association is likely due to a lack of power, especially in men. This result may be also due to the ethnic differences in linkage disequilibrium (LD) patterns, ethnic-specific association,

and gene/environmental interactions. Interestingly, 6 SNPs were monomorphic and SNP rs4836133 was triallelic in the Japanese population.

We found that rs206936 in the *NUDT3* gene was strongly associated with BMI and SFA only in women. rs206936 was weakly associated with increased VFA, but not with V/S ratio in women. Associations between rs206936, and SFA and VFA disappeared after adjustment with BMI, indicating that increased SFA and VFA are driven by increased BMI. A previously reported study did not show any association between rs206936 and VFA, SFA, and V/S ratio in both women and men [13]. The power was estimated to be 0.10 in men and 0.63 in women for VFA, 0.36 in men and 0.95 in women for SFA, and 0.19 in men and 0.05 in women for V/S ratio. Therefore, further studies comprising more subjects are required to investigate the association between rs206936 and SFA; however, rs206936 in the *NUDT3* gene is a good candidate for involvement in the accumulation of subcutaneous fat that leads to increased BMI.

SNP rs2112347 in the *POC5* gene ($P = 0.032$) and rs206936 in the *NUDT3* gene ($P = 0.018$), were not significantly, but weakly, associated with VFA in women. A very recent report indicated that rs2112347 is weakly associated with visceral fat tissue in women ($P = 0.002$) [13], and thus, rs2112347 may be associated with visceral fat accumulation. T-allele of rs2943650 in the *IRS1* gene is associated with decreased adiposity, especially reduced subcutaneous fat and the ratio of increased visceral fat to subcutaneous fat [22]. We observed that T-allele of rs2943650 was weakly associated with reduced VFA even after adjustment with BMI ($P = 0.041$). Thus, rs2943650 may also be associated with fat distribution in the Japanese.

Sexual dimorphism in fat distribution has been acknowledged long ago [27]. Under the sexual dimorphism in fat distribution, there are sex-specific genetic effects and genetic variance reported to be higher in women for the waist, hip and thigh circumference, and waist to hip ratio [32]. In our study, we observed the strong association of rs206936 in the *NUDT3* gene with SFA in women only. Thus, rs206936 in the *NUDT3* may be one of the sex-specific genetic variances. The SNPs in the *FTO* gene and rs7498665 in the *SH2B1* gene are associated with fat distribution, in men and women [14, 15, 20]. We have recently reported that SNPs in the *CYP17A1* and *NT5C2* genes were significantly associated with both reduced VFA and SFA in women, but not in men [33]. In addition, *LYPLAL1* rs4846567 has a stronger effect on the V/S ratio in women than in men [13, 16]. Therefore, some of the genetic variants may contribute the sex-specific genetic variances in fat distribution, possibly through sex steroids on transcription. Our studies [16, 33] and previous GWASs [10, 12, 13, 22] have highlighted the importance of understanding the underlying molecular mechanisms in sex differences in the regulation of body fat distribution.

The *NUDT3* rs206936 genotype did not show any association with metabolic disorders. This result was consistent with that of a previous report [30]. Visceral fat accumulation is more strongly linked to metabolic disorders than subcutaneous fat accumulation [1, 2, 28, 29]. The *NUDT3* rs206936 genotype showed strong association with SFA, and weak association with VFA and no association with V/S ratio.

NUDT3 is expressed in various tissues and has phosphatase activity directed against inorganic polyphosphates: diphosphoinositol pentakisphosphate and bis-diphosphoinositol tetrakisphosphate [34, 35]. Diphosphoinositol pentakisphosphate is reported to inhibit Akt signaling, which consists of a part of insulin singling system [36]. SNP rs206936 is situated in the intron 2 of the *NUDT3* gene and rs206936 may increase the *NUDT3* transcripts in the liver [21]. According to the HapMap database, rs206942 is in LD with rs206936 ($r^2 > 0.90$) and exists in the 3′-untranslated region of the *NUDT3* gene. rs206942 would influence the mRNA stabilization. Five SNPs (rs12662905, rs464553, rs3798560, rs206919 and rs6912971) in LD with rs206936 ($r^2 > 0.90$) exists in the 1st intron, suggesting these SNPs would alter the transcription level of *NUDT3* gene. The NUDT3 may be high in the subjects with risk allele and diphosphoinositol pentakisphosphate decreased catalyzed by NUDT3, resulting in the increased sensitivity of insulin signaling. Insulin stimulates lipid storage in adipose tissue, leading to obesity. Although the precise roles of NUDT3 and inorganic polyphosphates are unclear, NUDT3 may be involved in glucose and lipid metabolism through the regulation of intra-cellular signaling systems, thereby leading to the accumulation of fat.

In summary, we showed that *NUDT3* rs206936 is significantly associated with increased BMI and SFA in Japanese women. Our results suggest that the region containing the identified SNP in the *NUTD3* gene is specifically involved in increasing the relative amount of subcutaneous fat mass in women.