平成22・23年度　分担研究まとめ

分担課題名	樹状細胞および自己抗原ペプチドを用いた1型糖尿病発症抑制の検討（平成22年度分担研究）
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A．研究目的

劇症1型糖尿病の確実な診断マーカーを同定し、診断基準を確立する目的で、樹状細胞や自己抗原を介した機序の関与を検討することを目的とした。

B．研究方法

1型糖尿病のモデルマウスであるNODマウスに、NODマウスの骨髄由来樹状細胞を投与し、NODマウスでの糖尿病発症の抑制が可能かどうかを検討した。

次に、プレプロインスリンのシグナルペプチドL7-24をNODマウスに投与し、糖尿病発症抑制及び糖尿病状態からの改善が可能かどうかを検討した。

最後に、膵島自己抗原反応性CD8+T細胞クローン(NY8.3)T細胞受容体遺伝子を導入した8.3NODマウスより得られた自己反応性細胞傷害性CD8+T細胞を、in vitroで脾臓由来樹状細胞、自己抗原ペプチドIGRP、TGF-□及びレチノイン酸とともに培養することにより、自己反応性抑制性CD8+T細胞を樹立することが可能かどうか試みた。

C．研究結果

（実験1）

NODマウスの骨髄由来樹状細胞をNODマウスに投与したところ、有意にNODマウスにおける糖尿病発症を抑制した。

（実験2）

プレプロインスリンのシグナルペプチドL7-24は、有意にNODマウスにおける糖尿病発症を抑制した。また、糖尿病発症直後の投与により糖尿病状態からの改善が認められた。

（実験3）

自己反応性細胞傷害性CD8T細胞を自己反応性T細胞と共にNOD-Scidマウスに投与したところ、in vivoでNOD-Scidマウスへの糖尿病の移入を抑制した。また、in vitroでも自己反応性T細胞を抑制し、CD8陽性の自己反応性抑制性T細胞を樹立することが可能であった。

D．考察

本研究により、1型糖尿病発症における樹状細胞や自己抗原ペプチドの重要性と共に、それらを用いた1型糖尿病発症抑制の可能性が明らかになった。可能である事を示すとともに、CD8陽性抑制性T細胞が存在する事を明らかにした。

E．結論

自然免疫系の主役である樹状細胞と自己抗原ペプチドの1型糖尿病の発症、抑制及び予防における重要性が明らかとなった。自然免疫系が重要であることが示唆される劇症1型糖尿病の診断においても、患者様の樹状細胞を用いた細胞性免疫を応用したin vitro診断法の確立に自己抗原に加えて樹状細胞は極めて重要である事が示唆された。

Ⅳ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

該当なし

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
Yamaoka M, Kitamura T, Moriyama H, Shima Y, Haseda F, Okita K, Sakaguchi Y, Iwahashi H, Hanafusa T, Funahashi T, Nagata M, Otsuki M, Imagawa A, Shimomura I.	A case of long-standing autoimmune type 1 diabetes with common variable immunodeficiency.	Diabetol Int	3	50-53	2012
Hotta K, Hanafusa T et al	Genetic variations in the CYP17A1 and NT5C2 genes are associated with a reduction in visceral and subcutaneous fat areas in Japanese women.	Journal of Human Genetics	57	46-51	2012
Tsutsumi C, Imagawa A, Ikegami H, Makino H, Kobayashi T, Hanafusa T.	Class II HLA genotype in fulminant type 1 diabetes -a nationwide survey with reference to GAD antibodies.	J Diabetes Invest.	doi: 10.1111/j.2040-1124.2011.00139.x,		2011
Haseda F, Imagawa A, Murase-Mishiba Y, Sano H, Hirano-Kuwata S, Ueda H, Terasaki J, Hanafusa T.	Low CTLA-4 expression in CD4(+) helper T-cells in patients with fulminant type 1 diabetes.	Immunol Lett.	139(1-2)	80-86	2011
Hotta K, Hanafusa T et al	Computed tomography analysis of the association between the SH2B1 rs 7498665 single-nucleotide polymorphism and visceral fat area.	J Human Genet	53	716-719	2011
Tanimoto K, Hanafusa T et al	Intravascular and Intra-atrial Extension of Follicular Thyroid Carcinoma.	Thyroid	21(11)	1281-1282	2011
Tanimoto K, Hanafusa T et al	Hypopituitarism in a patient with transsphenoidal cephalocele: longitudinal changes in endocrinological abnormalities.	Endocrine J	53(3)	193-198	2011

Aida K, Nishida Y, Tanaka S, Maruyama T, Shimada A, Awata T, Suzuki M, Shimura H, Takizawa S, Ichijo M, Akiyama D, Furuya F, Kawaguchi A, Kaneshige M, Itakura J, Fujii H, Endo T, Kobayashi T.	RIG-I- and MDA5-Initiated Innate Immunity Linked With Adaptive Immunity Accelerates {beta}-Cell Death in Fulminant Type 1 Diabetes.	Diabetes	60	884-889	2011
Kobayashi T, Nishida Y, Tanaka S, Aida K.	Pathological changes in the pancreas of fulminant type 1 diabetes and slowly progressive insulin-dependent diabetes mellitus (SPIDDM): innate immunity in fulminant type 1 diabetes and SPIDDM	Diabetes Metab Res Rev.	27	965-970	2011
Li H, Ikegami H et al.	Association of genetic variation in FTO with risk of obesity and type 2 diabetes in up to 96,551 East and South Asians.	Diabetologia	55	981-995	2012
Cho YS, Ikegami H et al.	Meta-analysis of genome-wide association studies identifies 8 new loci for type 2 diabetes in East Asians.	Nature Genet	44	67-72	2012
Ikegami H, Noso S, Babaya N, Kawabata Y	Genetics and pathogenesis of type 1 diabetes: prospects for prevention and intervention	J Diabetes Invest	2(6)	415-420	2011
Moriguchi M, Noso S, Kawabata Y, Yamauchi T, Harada T, Komaki K, Babaya N, Hiromine Y, Ito H, Yamagata S, Murata K, Higashimoto T, Park C, Yamamoto A, Ohno Y, Ikegami H	Clinical and genetic characteristics of patients with autoimmune thyroid disease with anti-islet autoimmunity.	Metabolism	60	761-766	2011
Nishida N, Mawatari Y, Sageshima M, Tokunaga K	Highly Parallel and Short-Acting Amplification with Locus-Specific Primers to Detect Single Nucleotide Polymorphisms by the DigiTag2 Assay.	PLoS One	7(1)	e29967	2012

Yamashita H, Awata T, Kawasaki E, Ikegami H, Tanaka S, Maruyama T, Shimada A, Nakanishi K, Takahashi K, Kobayashi T, Kawabata Y, Miyashita Y, Kurihara S, Morita-Ohkubo T, Katayama S; Japanese Study Group on Type 1 Diabetes Genetics.	Analysis of the HLA and non-HLA susceptibility loci in Japanese type 1 diabetes.	Diabetes Metab Res Rev	27(8)	844-848	2011
Kawasaki E, Nakamura K, Kuriya G, Satoh T, Kobayashi M, Kuwahara H, Abiru N, Yamasaki H, Matsuura N, Miura J, Uchigata Y, Eguchi K.	Zinc transporter 8 autoantibodies in fulminant, acute-onset, and slow-onset patients with type 1 diabetes.	Diabetes Metab Res Rev	27	895-898	2011
Nakamura K, Kawasaki E, Imagawa A, Awata T, Ikegami H, Uchigata Y, Kobayashi T, Shimada A, Nakanishi K, Makino H, Maruyama T, Hanafusa T; Research Committee on Type 1 Diabetes of the Japan Diabetes Society.	Type 1 diabetes and interferon therapy: a nationwide survey in Japan.	Diabetes Care.	34(9)	2084-2089	2011
西村保一郎	統計ソフトRによるロジスティック回帰分析	大阪医科大学雑誌	70-3	7-11	2011
今川彰久、花房俊昭	劇症1型糖尿病	臨床病理	58(3)	216-224	2010
Sano H, Hanafusa T et al.	Exendin-4, a glucagon-like peptide-1 receptor agonist, suppresses pancreatic beta-cell destruction induced by encephalomyocarditis virus.	Biochem Biophys Res Commun	404(3)	756-61.	2011
Uno S, Hanafusa T, et al.	Expression of chemokines, CXC chemokine ligand 10 (CXCL10) and CXCR3 in the inflamed islets of patients with recent-onset autoimmune type 1 diabetes.	Endocr J	57(11)	991-6	2010

Shibasaki S, Imagawa A, Terasaki J, Hanafusa T.	Endogenous insulin secretion even at a very low level contributes to the stability of blood glucose control in fulminant type 1 diabetes.	J Diabetes Invest	1(6)	283-285.	2010
Koga M, Hanafusa T, et al.	Correlation of glycated albumin but not hemoglobin A1C with endogenous insulin secretion in fulminant type 1 diabetes mellitus.	J Diabetes Invest	1(6)	:279-282.	2010
Koga M, Hanafusa T, et al.	Serum 1,5-anhydroglucitol levels in patients with fulminant type 1 diabetes are lower than those in patients with type 2 diabetes.	Clin Biochem.	43(15)	1265-7	2010
Shibasaki S, Hanafusa T, et al.	Expression of toll-like receptors in the pancreas of recent-onset fulminant type 1 diabetes.	Endocr J.	57(3)	211-9	2010
Noso S, Kataoka K, Kawabata Y, Babaya N, Hiromine Y, Yamaji K, Fujisawa T, Aramata S, Kudo T, Takahashi S, Ikegami H	Insulin transactivator MafA regulates intra-thymic expression of insulin and affects susceptibility to type 1 diabetes.	Diabetes	59 (10)	2579-87	2010
Babaya N, Fujisawa T, Nojima K, Itoi-Babaya M, Yamaji K, Yamada K, Kobayashi M, Ueda H, Hiromine Y, Noso S, Ikegami H	Direct evidence for susceptibility genes for type 2 diabetes on mouse chromosomes 11 and 14.	Diabetologia	53	1362-1371	2010
西田奈央、徳永勝士	SNP解析	バイオチップ実用化ハンドブック		139-146	2010
徳永勝士	ゲノムワイド関連解析による疾患感受性遺伝子・薬剤応答性遺伝子の探索	ファルマシア	46	421-431	2010
Kawasaki E, Nakamura K, Kuriya G, Satoh T, Kobayashi M, Kuwahara H, Abiru N, Yamasaki H, Matsuura N, Miura J, Uchigata Y, Eguchi K.	Differences in the humoral autoreactivity to zinc transporter 8 between childhood- and adult-onset type 1 diabetes in Japanese patients.	Clin Immunol	138	146-153	2011

Nakamura K, Kawasaki E, Abiru N, Jo O, Fukushima K, Satoh T, Kuriya G, Kobayashi M, Kuwahara H, Yamasaki H, Ide T, Eguchi K.	Trajectories of ant-islet autoantibodies before development of type 1 diabetes in interferon-treated hepatitis C patients. Case reports and a literature review.	Endocr J	57	947-951	2010
Horie I, Kawasaki E, Shimomura A, Satoh T, Ueki I, Kuwahara H, Ando T, Abiru N, Usa T, Eguchi K.	Emergence of anti-islet autoantibodies in Japanese patients with type 1 diabetes.	Endocr J	57	623-628	2010
Arai T, Moriyama H, Shimizu M, Sasaki H, Kishi M, Okumachi Y, Yasuda H, Hara K, Yokono K, Nagata M.	Administration of a determinant of preproinsulin can induce regulatory T cells and suppress anti-islet autoimmunity in NOD	Clin Immunol.	136(1)	74-82	2010
Kishi M, Yasuda H, Abe Y, Sasaki H, Shimizu M, Arai T, Okumachi Y, Moriyama H, Hara K, Yokono K, Nagata M	Regulatory CD8+ T cells induced by exposure to all-trans retinoic acid and TGF-b suppress autoimmune diabetes.	Biochem Biophys Res Commun.	394(1)	228-32	2010
Yasuda H, Nagata M, Hara K, Moriyama H, Yokono K.	Biguanide, but not thiazolidinedione, improved insulin resistance in Werner syndrome.	J Am Geriatr Soc.	58(1)	181-2	2010
小林寛和、安田尚史、河野泰博、明嵜太一、森山啓明、原　賢太、櫻井　孝、永田正男	１型糖尿病の経過中に食道アカラシアを合併した多腺性自己免疫症候群の一例	糖尿病	53(12)	829-833	2010
中村　晃、安田尚史、明嵜太一、原　賢太、永田正男、横野浩一	膵腎同時移植後に肺クリプトコッカス症を発症した１型糖尿病の１例	糖尿病	53(8)	607-612	2010

Ⅴ．研究成果の刊行物・別刷

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CASE REPORT

A case of long-standing autoimmune type 1 diabetes with common variable immunodeficiency

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Abstract Several lines of evidence have suggested that pancreatic β-cell destruction is caused by inflammatory cellular responses mediated by T lymphocytes in individuals with type 1A diabetes. B lymphocytes, which play an important role in the production of autoantibodies to β-cell antigens such as insulin, glutamic acid decarboxylase (GAD) or insulinoma associated antigen 2 (IA-2) in type 1A diabetes, are also known as professional antigen-presenting cells and T-lymphocyte activators. Here, we report a case of long-standing autoimmune type 1 diabetes with common variable immunodeficiency, which is known as a functional deficiency of B lymphocytes. A 51-year-old man was admitted to our hospital because of hyperglycemia. He had suffered from frequent bacterial infections from early childhood. At 16 years old, he was diagnosed with common variable immunodeficiency. At age 27, he experienced sudden-onset diabetic ketosis and was diagnosed with type 1 diabetes. Enzyme-linked immunospot (ELISPOT) assay recently revealed that interferon-γ-producing T lymphocytes but not interleukin 4-producing T lymphocytes, which react with GAD and insulin $B_{1\text{-}18}$, were present at increased levels in his peripheral blood at 51 years old. This case represents the longest reported interval between onset of type 1 diabetes and confirmation of cell-mediated autoimmunity against pancreatic β-cells in a patient with common variable immunodeficiency.

Keywords IDDM · CVID · ELISPOT · GAD · IA-2

Introduction

Several lines of evidence have suggested that pancreatic β-cell destruction is caused by inflammatory cellular responses mediated by T lymphocytes in individuals with type 1A diabetes [1, 2]. B lymphocytes, which play an important role in the production of autoantibodies to β cell antigens such as insulin, GAD or IA-2 in type 1A diabetes patients, are also known as professional antigen-presenting cells and T-lymphocyte activators.

Here, we report a case of long-standing autoimmune type 1 diabetes with common variable immunodeficiency (CVID), which is a functional deficiency of B lymphocytes.

Case report

In 2009, a 51-year-old man was admitted to our hospital because of hyperglycemia. He had suffered from frequent bacterial infections from early childhood. At 16 years old, he was diagnosed with CVID. At age 27, he had sudden-

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onset diabetic ketosis and was diagnosed with type 1 diabetes. At admission to our hospital, his blood glucose level was 306 mg/dl, and urinary C peptide excretion was reduced to 8.7 μg/day. The serum IgG level was as low as 340 mg/dl (normal range 870–1,700 mg/dl), the IgA level was 57 mg/dl (normal range 110–410 mg/dl), and the IgM level was 31 mg/dl (normal range 35–220 mg/dl). Subcutaneous injection of insulin was started. He has been treated with monthly intravenous injections of γ-globulin (5,000 mg) since the age of 45. Immunoglobulin supplementation led to a marked reduction in the number of infections. Islet cell antibodies (ICA) and GAD antibodies were not detected either at 46 years old or on admission. IA-2 antibody, insulin autoantibodies (IAA), antinuclear antibody, thyroid-stimulating hormone (TSH) receptor antibody and thyroglobulin antibody tests were also negative.

Laboratory data on admission revealed normal blood count and blood chemistry. However, the CD19+ B-lymphocyte level (139/μl) but not the CD3+ T-lymphocyte level (1,160/μl) was decreased. The CD4/CD8 ratio had decreased to 30.5/46.0 (0.66). The HLA haplotypes of *DRB1-DQB1* were **04:05-*04:01* and **11:01-*03:01*. A glucagon tolerance test revealed the complete loss of endogenous insulin secretion capacity; the serum C-peptide concentrations before and 6 min after injection were undetectable (below 0.01 ng/ml).

We measured the responses of pancreatic β-cell-reactive peripheral T lymphocytes using an immunoglobulin-free enzyme-linked immunospot (ELISPOT) assay as described previously [3]. The mean number of interferon (IFN)-γ spots reactive to GAD_{65} and insulin $B_{1\text{-}18}$ peptide was 7.5 and 3.5, respectively, in a duplicate assay. Interleukin (IL)-4 spots reactive to those peptides were not detected. Neither IFN-γ spots nor IL-4 spots reacted to insulin $B_{9\text{-}23}$, $B_{10\text{-}24}$, $A_{1\text{-}15}$ and $L_{7\text{-}23}$. To compare the positivity among the other diabetic patients and control subjects in ELISPOT assay, the mean number of antigen-stimulated IFN-γ spots reactive to GAD_{65} was plotted after subtracting the background (T cells only). A significant IFN-γ response to the GAD_{65} peptide was observed in this patient (Fig. 1a). Data for other patients with type 1A diabetes and type 2 diabetes and for healthy controls were taken from our previous report [3].

Two-color flow cytometric analysis revealed decreased numbers of $CD10^-$ $CD19^+$ cells and $CD10^-$ $CD22^+$ cells (mature B lymphocytes) (Fig. 1b, c). However, the numbers of $CD10^+$ $CD19^-$ cells and $CD10^+$ $CD22^-$ cells (immature B lymphocytes) were not increased (Fig. 1b, c). The reference values of our methods were less than 1.0% for $CD10^+$ cells, 5.0–24.0% for $CD19^+$ cells and 2.0–17.0% for $CD22^+$ cells. $CD4^+$ $FoxP3^+$ regulatory T cells were 2.5% in this patient, while they were 3.6 (1.2–5.1)% [median (range)] in 20 healthy individuals [16].

Discussion

We report the first case of established T cell immunity in an autoimmune type 1 (type 1A) diabetes patient with CVID. Islet autoantibodies were not detected; however, an ELISPOT assay, a useful tool to detect T-lymphocyte-mediated autoimmunity directly with good reproducibility in type 1 diabetes patients [3, 4], revealed GAD- and insulin $B_{1\text{-}18}$-reactive Th1 cells, but not GAD- and insulin $B_{1\text{-}18}$-reactive Th2 cells among the peripheral lymphocytes in this patient. T-lymphocyte reactivity specific to beta cell

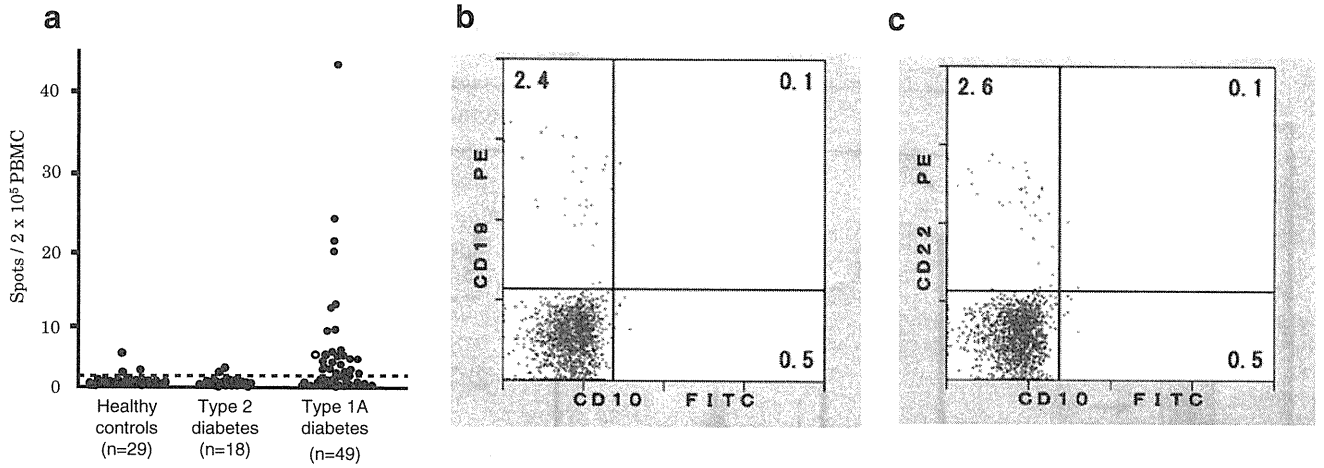

Fig. 1 **a** IFN-γ spots reactive to GAD_{65} in ELISPOT assays for subjects with type 1A diabetes or type 2 diabetes and for normal control subjects. The *open circle* represents our patient. Other data were taken from reference 3. Two-color flow cytometric analysis of our patient's PBMCs. A decreased number of $CD10^-$ $CD19^+$ cells and a normal number of $CD10^+$ $CD19^-$ cells (**b**) and a decreased number of $CD10^-$ $CD22^+$ cells and a normal number of $CD10^+$ $CD22^-$ cells (**c**) are shown

antigens of insulin B_{1-18} suggested the presence of a beta-cell-specific immune response. Higashide et al. [17] have reported that insulin B_{1-15} reactive Th1 cells are present in 6 of 18 recent-onset type 1 diabetic patients by ELISPOT assay, also suggesting the presence of insulin B_{1-18}-reactive Th1 cells in this patient indicates a long-lasting autoimmune response rather than acquired response induced by the long-lasting insulin treatment. There have already been several reports of probable type 1 diabetes with CVID, and ICA were detected in one of these patients. However, these patients did not have T lymphocyte immune reactivity to β cells at all [5–9].

CVID is a primary immune disorder characterized by hypogammaglobulinemia, antibody deficiency and recurrent infections [10]. This patient had (1) repeated infections in his childhood but not in his babyhood, (2) low levels of IgM, IgG and IgA in sera, (3) a normal number of T lymphocytes and (4) decreased but not absent B lymphocytes in his peripheral blood.

Our examination suggested that both the number and function of B lymphocytes were reduced in this patient. Laboratory data revealed a decreased $CD19^+$ B-lymphocyte level (139/μl). Flow cytometric analysis revealed no insufficient maturation of B lymphocytes in this patient. The functional deficiency of B lymphocytes was not directly observed; however, the history of repeated infections and improvement resulting from γ-globulin supplementation suggests non-specific functional loss of B lymphocytes. The lack of islet-related autoantibodies in spite of the positive reaction for his T lymphocytes against islet autoantigens might also indicate the reduction of B-lymphocyte function. On the other hand, the number of T lymphocyte including regulatory T cells, was normal in this patient.

This patient suffers from type 1A diabetes. This fact might indicate that B-lymphocyte insufficiency is not essential to the development of human type 1A diabetes despite the evidence in non-obese diabetic (NOD) mice, a rodent model [11–13]. The number of B lymphocytes in human insulitis lesions is low [2]. The effect of anti-CD20 therapy was limited to the patients with established autoimmune type 1 diabetes [14]. Type 1 diabetes has even been reported in a patient with X-linked severe agammaglobulinemia [15]. All of these findings suggest that B lymphocytes are not necessary to develop autoimmune β cell destruction in humans. Our present case with type 1A diabetes and CVID supports this concept for human type 1A diabetes. In addition, our patient had a positive reaction of T lymphocytes to islet autoantigens even 24 years after the onset of type 1 diabetes. These results might indicate that B-lymphocyte-mediated immunodeficiency was able to maintain anti-β cell autoimmunity long after disease onset.

Autoimmune diseases are more frequent in CVID patients than in general population [18, 19]. However, the prevalence of type 1 diabetes in CVID patients is not well documented. The established diagnosis of type 1 diabetes is sometimes difficult in CVID patients because islet autoantibodies are negative despite the presence of islet autoimmunity shown in the present case. It may underrepresent the prevalence of type 1 diabetes in CVID patients.

In conclusion, this case represents the longest reported interval between onset of type 1 diabetes and confirmation of cell-mediated autoimmunity against pancreatic β-cells in a patient with CVID.

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References

1. Eisenbarth GS, Polonsky KS, Buse JB. Type 1 diabetes mellitus. In: Larsen PR et al., editors. Williams textbook of endocrinology. 11th ed. Philadelphia: Saunders; 2008. p. 1391–1416.
2. Itoh N, Hanafusa T, Miyazaki A, Miyagawa J, Yamagata K, Yamamoto K, Waguri M, Imagawa A, Tamura S, Inada M, Kawata S, Tarui S, Kono N, Matsuzawa Y. Mononuclear cell infiltration and its relation to the expression of major histocompatibility complex antigens and adhesion molecules in pancreas biopsy specimens from newly diagnosed insulin-dependent diabetes mellitus patients. J Clin Invest. 1993;92:2313–22.
3. Kotani R, Nagata M, Imagawa A, Moriyama H, Yasuda H, Miyagawa J, Hanafusa T, Yokono K. T lymphocyte response against pancreatic beta cell antigens in fulminant Type 1 diabetes. Diabetologia. 2004;47:1285–91.
4. Nagata M, Kotani R, Moriyama H, Yokono K, Roep BO, Peakman M. Detection of autoreactive T cells in type 1 diabetes using coded autoantigens and an immunoglobulin-free cytokine ELISPOT assay: report from the fourth immunology of diabetes society T cell workshop. Ann N Y Acad Sci. 2004;1037:10–5.
5. Moffitt JE, Guill MF, Leffell MS, Ades EW, Burek CL, Lobel SA, Hoffman WH. Type I diabetes in an adolescent with common variable immunodeficiency. J Allergy Clin Immunol. 1989;84:191–6.
6. Metin A, Tezcan I, Ozyürek H. IDDM in an adolescent patient with common variable immunodeficiency. Diabetes Care. 1997;20:677–8.
7. Iglesias P, Ferreira A, Diez JJ. Common variable immunodeficiency in adult woman with IDDM. Diabetes Care. 1998;21:1029.
8. Lopez Cruz MC, Martin Mateos MA, Giner Munoz MT, Plaza Martin AM, Sierra Martinez JI. Common variable immunodeficiency, insulin-dependent diabetes mellitus and celiac disease. Allergol Immunopathol (Madr). 2000;28:323–7.
9. Topaloglu AK, Yuksel B, Yilmaz M, Mungan NO, Guneser S, Ozer G. Coexistence of common variable immunodeficiency and autoimmune polyglandular syndrome type 2. J Pediatr Endocrinol Metab. 2001;14:565–6.
10. International Union of Immunological Societies. Primary immunodeficiency diseases, report of an IUIS scientific committee. Clin Exp Immunol. 1999;118(Suppl 1):1–28.
11. Serreze DV, Chapman HD, Varnum DS, Hanson MS, Reifsnyder PC, Richard SD, Fleming SA, Leiter EH, Shultz LD.

B lymphocytes are essential for the initiation of T cell-mediated autoimmune diabetes: analysis of a new "speed congenic" stock of NOD.Ig mu null mice. J Exp Med. 1996;184:2049–53.
12. Noorchashm H, Noorchashm N, Kern J, Rostami SY, Barker CF, Naji A. B-cells are required for the initiation of insulitis and sialitis in nonobese diabetic mice. Diabetes. 1997;46:941–6.
13. Hu CY, Rodriguez-Pinto D, Du W, Ahuja A, Henegariu O, Wong FS, Shlomchik MJ, Wen L. Treatment with CD20-specific antibody prevents and reverses autoimmune diabetes in mice. J Clin Invest. 2007;117:3857–67.
14. Pescovitz MD, Greenbaum CJ, Krause-Steinrauf H, Becker DJ, Gitelman SE, Goland R, Gottlieb PA, Marks JB, McGee PF, Moran AM, Raskin P, Rodriguez H, Schatz DA, Wherrett D, Wilson DM, Lachin JM, Skyler JS. Type 1 Diabetes TrialNet Anti-CD20 Study Group. Rituximab, B-lymphocyte depletion, and preservation of beta-cell function. N Engl J Med. 2009;361: 2143–52.
15. Martin S, Wolf-Eichbaum D, Duinkerken G, Scherbaum WA, Kolb H, Noordzij JG, Roep BO. Development of type 1 diabetes despite severe hereditary B-lymphocyte deficiency. N Engl J Med. 2001;345:1036–40.
16. Haseda F, Imagawa A, Murase-Mishiba Y, Sano H, Hirano-Kuwata S, Ueda H, Terasaki J, Hanafusa T. Low CTLA-4 expression in $CD4^+$ helper T-cells in patients with fulminant type 1 diabetes. Immunol Lett. 2011;139:80–6.
17. Higashide T, Kawamura T, Nagata M, Kotani R, Kimura K, Hirose M, Inada H, Niihira S, Yamano T. T cell epitope mapping study with insulin overlapping peptides using ELISPOT assay in Japanese children and adolescents with type 1 diabetes. Pediatr Res. 2006;59:445–50.
18. Cunningham-Rundles C, Bodian C. Common variable immunodeficiency: clinical and immunological features of 248 patients. Clin Immunol. 1999;92:34–48.
19. Quinti I, Soresina A, Spadaro G, Martino S, Donnanno S, Agostini C, Claudio P, Franco D, Maria Pesce A, Borghese F, Guerra A, Rondelli R, Plebani A, Italian Primary Immunodeficiency Network. Long-term follow-up and outcome of a large cohort of patients with common variable immunodeficiency. J Clin Immunol. 2007;27:308–16

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ORIGINAL ARTICLE

Genetic variations in the *CYP17A1* and *NT5C2* genes are associated with a reduction in visceral and subcutaneous fat areas in Japanese women

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Visceral fat accumulation has an important role in increasing the morbidity and mortality rates, by increasing the risk of developing several metabolic disorders, such as type 2 diabetes, dyslipidemia and hypertension. New genetic loci that are associated with increased systolic and diastolic blood pressures have been identified by genome-wide association studies in Caucasian populations. This study investigates whether single nucleotide polymorphisms (SNPs) that confer susceptibility to high blood pressure are also associated with visceral fat obesity. We genotyped 1279 Japanese subjects (556 men and 723 women) who underwent computed tomography for measuring the visceral fat area (VFA) and subcutaneous fat area (SFA) at the following SNPs: *FGF5* rs16998073, *CACNB2* rs11014166, *C10orf107* rs1530440, *CYP17A1* rs1004467, *NT5C2* rs11191548, *PLEKHA7* rs381815, *ATP2B1* rs2681472 and rs2681492, *ARID3B* rs6495112, *CSK* rs1378942, *PLCD3* rs12946454, and *ZNF652* rs16948048. In an additive model, risk alleles of the *CYP17A1* rs1004467 and *NT5C2* rs11191548 were found to be significantly associated with reduced SFA (P=0.00011 and 0.0016, respectively). When the analysis was performed separately in men and women, significant associations of rs1004467 (additive model) and rs11191548 (recessive model) with reduced VFA (P=0.0018 and 0.0022, respectively) and SFA (P=0.00039 and 0.00059, respectively) were observed in women, but not in men. Our results suggest that polymorphisms in the *CYP17A1* and *NT5C2* genes influence a reduction in both visceral and subcutaneous fat mass in Japanese women.
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Keywords: computed tomography; *CYP17A1*; Japanese subjects; *NT5C2*; sexual dimorphism; subcutaneous fat area; visceral fat area

INTRODUCTION

Metabolic syndrome is a combination of multiple risk factors, including central obesity, impaired glucose tolerance, dyslipidemia and hypertension, which increases cardiovascular disease morbidity and mortality.[1] Several studies have indicated that the intra-abdominal adipose tissue has a central role in metabolic syndrome, as the

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accumulated visceral adipose tissue leads to alterations in the plasma levels of adipocytokines, resulting in the development of dyslipidemia, hypertension and insulin resistance.[2,3] Intra-abdominal fat accumulation (central adiposity) is determined by waist circumference, waist–hip ratio, biological impedance or the visceral fat area (VFA) measured using computed tomography.[1,4,5] There is abundant evidence that body fat distribution is influenced by genetic loci.[6–8] Individual variation in waist–hip ratio is heritable, with heritability estimates ranging from 22 to 61%. Recent genome-wide association studies (GWAS) showed that genetic loci were associated with waist circumference and waist–hip ratio in the Caucasian population.[9,10] We previously reported that the rs1558902 and rs1421085 genotypes of the fat mass- and obesity-associated gene (*FTO*) were significantly associated with VFA, as well as with the subcutaneous fat area (SFA) and body mass index (BMI) in the Japanese population.[11]

Recent progress in GWAS has increased the number of known genetic susceptibility loci for obesity.[12–16] We investigated the association between the single nucleotide polymorphisms (SNPs) underlying susceptibility to obesity and fat distribution (as determined by computed tomography), and found that rs7498665 in the SH2B adaptor protein 1 (*SH2B1*) gene was associated with VFA, uncovering the genetic background of central obesity.[17]

GWAS, and meta-analysis of GWAS, have identified various disease-associated genetic variations.[18] Hypertension is one of the risk factors of metabolic syndrome and is considerably related to central obesity. Obesity-associated allele of rs1558902 and rs1421085 in the *FTO* gene were associated with hypertension, but not that of rs7498665 in the *SH2B1* gene in the Japanese population.[19] The genetic variations associated with hypertension have been identified by GWAS.[20,21] In this study, we investigate whether the recently reported hypertension-related loci are also associated with VFA, which is another important factor responsible for metabolic syndrome.

MATERIALS AND METHODS

Study subjects

We enrolled 1279 Japanese subjects from outpatient clinics; these patients agreed to undergo computed tomography testing (in the supine position) to determine VFA and SFA values at the umbilical level (L4–L5), as previously reported.[17] Both VFA and SFA values were calculated using the FatScan software program (N2system, Osaka, Japan).[22] The patients visited the hospitals to undergo treatment for obesity and/or metabolic abnormalities, such as hypertension, dyslipidemia and type 2 diabetes. Patients with secondary obesity and obesity-related hereditary disorders were excluded from this study. Patients with disease (such as cancer, and renal, heart and hepatic failure), or under treatment (such as corticosteroid and chemotherapy) that strongly affects body weight, were also excluded. Athletes were also excluded from this study. Clinical data were recorded at the first visit to the hospital. The clinical characteristics of the subjects are summarized in Table 1. Metabolic syndrome and metabolic abnormalities were diagnosed according to the criteria released by the Japanese Committee for the Diagnostic Criteria of Metabolic Syndrome in April 2005.[4,5] Written informed consent was obtained from each subject, and the protocol was approved by the ethics committee of each institution and by that of 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 12 SNPs that were previously identified as susceptibility loci for hypertension by GWAS in Caucasian populations,[20,21] and constructed Invader probes (Third Wave Technologies, Madison, WI, USA) for each. The 12 selected SNPs were as follows: rs16998073 in the fibroblast growth factor 5 (*FGF5*) gene; rs11014166 in the calcium channel, voltage-dependent, β-2 subunit (*CACNB2*) gene; rs1530440 in the chromosome 10 open reading frame 107 (*C10orf107*) gene; rs1004467 in the cytochrome P450, family 17, subfamily A, polypeptide 1 (*CYP17A1*) gene; rs11191548 in the 5′-nucleotidase, cytosolic II (*NT5C2*) gene; rs381815 in the pleckstrin homology domain containing, family A member 7 (*PLEKHA7*) gene; rs2681472 and rs2681492 in the ATPase, Ca^{2+} transporting, plasma membrane 1 (*ATP2B1*) gene; rs6495112 in the AT-rich interactive domain 3B (BRIGHT-like) (*ARID3B*) gene; rs1378942 in the c-src tyrosine kinase (*CSK*) gene; rs12946454 in the phospholipase C, delta 3 (*PLCD3*) gene; and rs16948048 in the zinc finger protein 652 (*ZNF652*) gene. The SNPs were genotyped using Invader assays, as previously described.[23] The success rate of these assays was >99.0%.

Table 1 Clinical characteristics of the subjects

	Men	*Women*	*Total*
n	556	723	1279
Age (years)	49.4±12.2	52.2±11.3	51.0±11.8
BMI (kg m^{-2})	30.2±6.1	28.1±5.3	29.0±5.8
VFA (cm^2)	155.3±67.7	99.8±53.6	123.9±66.1
SFA (cm^2)	206.7±108.6	241.6±97.2	226.5±103.7
Waist circumference (cm)	97.5±11.3	91.8±10.3	94.2±11.1
Prevalence of metabolic disease			
Dyslipidemia	293 (53%)	244 (34%)	537 (42%)
Hypertension	379 (68%)	452 (63%)	831 (65%)
Impaired fasting glucose	177 (32%)	176 (24%)	353 (28%)
Metabolic syndrome	248 (45%)	162 (22%)	410 (32%)

Abbreviations: BMI, body mass index; SFA, subcutaneous fat area; VFA, visceral fat area.
Data are represented as mean ± s.d.

Statistical analysis

For the additive model, we coded the genotypes as 0, 1 or 2 depending on the number of copies of the risk alleles. For the recessive model, homozygosity with the risk allele was coded as 1 and the others were coded as 0. Risk alleles refer to the hypertension-associated alleles, according to previous reports.[20,21] Multiple linear regression analyses were performed to test the independent effect of the risk alleles on BMI, VFA and SFA, by taking into account the effects of other variables (that is, age and gender) that were assumed to be independent of the effect of each SNP. The values of BMI, VFA and SFA were logarithmically transformed before performing the multiple linear regression analysis. Differences in the quantities of anthropometric parameters among the different genotypes were assessed by the analysis of covariance, by taking into account the effects of other variables (that is, age and/or institute). Hardy–Weinberg equilibrium was assessed using the χ^2-test.[24] To test SNP×SNP epistasis, we used a linear regression model for each SNP1 and SNP2, and fit the model in the form of $Y=\beta_0+\beta_1\times\mathrm{SNP1}+\beta_2\times\mathrm{SNP2}+\beta_3\times\mathrm{SNP1}\times\mathrm{SNP2}+\beta_4\times\mathrm{age}+\beta_5\times\mathrm{gender}$. Although we collected the samples at the region of Hondo (Kanto, Kinki, Chugoku and Kyushu; Supplementary Table 1), we performed Wright's F-statistics[25] to evaluate the difference in the population structures of our sample using randomly selected 31 SNPs. We divided our samples into two groups (SFA >208 cm^2 and ⩽208 cm^2). Median of SFA (208 cm^2) was used as a cut-off value. The results indicated that the population structure of the two groups were almost the same in view of a very small F_{ST} value between both the groups (mean F_{ST}=0.00023). Statistical analysis was performed using R software (http://www.r-project.org/). *P*-values were assessed with a Bonferroni correction and $P<0.0042$ (0.05/12) was considered statistically significant.

RESULTS

The clinical characteristics and genotypes of the subjects are shown in Tables 1 and 2, respectively. All the SNPs were in Hardy–Weinberg equilibrium and the minor allele frequencies did not diverge from those reported in the HapMap database. The BMI, VFA and SFA values for each SNP genotype are reported in Table 3. Multiple linear regression analyses of the anthropometric parameters with respect to the 12 analyzed SNPs are shown in Table 4. The A-allele of rs1004467

Table 2 Genotypic characteristics of the subjects

SNP ID	*CHR*	*Position (Build 36.3)*	*Nearby gene*	*Allele 1/2*	*BP-associated allele*	*Genotype*	*HWE P-value*
rs16998073	4	81 403 365	*FGF5*	T/A	T	120/514/644	0.24
rs11014166	10	18 748 804	*CACNB2*	T/A	A	4/124/1151	0.73
rs1530440	10	63 194 597	*C10orf107*	T/C	C	30/296/953	0.22
rs1004467	10	104 584 497	*CYP17A1*	A/G	A	559/567/153	0.62
rs11191548	10	104 836 168	*NT5C2*	T/C	T	675/504/100	0.66
rs381815	11	16 858 844	*PLEKHA7*	C/T	T	842/381/56	0.13
rs2681472	12	88 533 090	*ATP2B1*	A/G	A	546/562/171	0.17
rs2681492	12	88 537 220	*ATP2B1*	C/T	T	168/561/549	0.19
rs6495112	15	72 619 851	*ARID3B*	A/C	A	530/575/173	0.39
rs1378942	15	72 864 420	*CSK*	A/C	C	49/410/817	0.78
rs12946454	17	40 563 647	*PLCD3*	T/A	T	34/343/901	0.84
rs16948048	17	44 795 465	*ZNF652*	G/A	G	18/326/935	0.08

Abbreviations: BP, blood pressure; CHR, chromosome; HWE, Hardy–Weinberg equilibrium.

Table 3 Mean BMI, VFA and SFA for 12 blood pressure risk variants

		Mean ± s.d.								
		BMI (kg m^{-2})			*VFA (cm^2)*			*SFA (cm^2)*		
		Genotype			*Genotype*			*Genotype*		
SNP ID	*Nearby gene*	*11*	*12*	*22*	*11*	*12*	*22*	*11*	*12*	*22*
rs16998073	*FGF5*	28.8 ± 4.7	29.0 ± 5.8	29.0 ± 6.0	126.2 ± 66.1	121.6 ± 66.5	125.3 ± 65.9	227.4 ± 98.3	224.5 ± 111.0	227.7 ± 98.7
rs11014166	*CACNB2*	27.0 ± 2.7	29.6 ± 6.1	28.9 ± 5.8	123.4 ± 82.2	136.7 ± 68.2	122.5 ± 65.8	178.6 ± 35.8	233.7 ± 106.4	225.8 ± 103.6
rs1530440	*C10orf107*	30.8 ± 6.5	28.6 ± 5.4	29.1 ± 5.9	129.8 ± 63.1	120.2 ± 66.4	124.9 ± 66.2	236.4 ± 119.2	223.0 ± 91.4	227.2 ± 106.9
rs1004467	*CYP17A1*	28.4 ± 5.6	29.5 ± 6.1	29.4 ± 5.2	117.5 ± 64.9	130.6 ± 68.5	122.5 ± 59.3	215.5 ± 92.7	231.4 ± 111.5	247.9 ± 107.9
rs11191548	*NT5C2*	28.6 ± 5.8	29.5 ± 5.9	28.9 ± 5.1	119.2 ± 65.3	130.9 ± 68.6	120.5 ± 55.7	217.2 ± 96.0	238.5 ± 113.2	228.1 ± 98.8
rs381815	*PLEKHA7*	29.2 ± 5.9	28.7 ± 5.7	27.8 ± 4.3	124.1 ± 64.2	124.3 ± 71.5	117.9 ± 55.9	229.4 ± 105.9	221.5 ± 101.6	215.3 ± 83.1
rs2681472	*ATP2B1*	29.2 ± 5.8	28.8 ± 5.4	29.0 ± 7.0	127.1 ± 67.5	121.5 ± 64.8	121.8 ± 65.8	227.4 ± 100.4	223.5 ± 100.3	233.1 ± 123.6
rs2681492	*ATP2B1*	29.0 ± 7.1	28.7 ± 5.2	29.3 ± 5.9	121.9 ± 66.3	121.4 ± 64.8	127.0 ± 67.4	234.6 ± 123.9	221.9 ± 98.3	228.7 ± 102.3
rs6495112	*ARID3B*	28.9 ± 5.8	29.0 ± 5.7	29.3 ± 6.2	122.5 ± 63.4	125.0 ± 69.2	124.9 ± 64.3	223.7 ± 106.5	229.5 ± 102.6	225.1 ± 99.2
rs1378942	*CSK*	28.0 ± 4.2	28.9 ± 6.1	29.1 ± 5.7	110.0 ± 63.3	121.8 ± 62.4	125.6 ± 67.6	222.9 ± 84.5	225.9 ± 104.3	227.0 ± 104.7
rs12946454	*PLCD3*	30.1 ± 8.2	28.6 ± 5.0	29.1 ± 5.9	137.2 ± 80.0	123.0 ± 67.4	123.7 ± 65.1	254.4 ± 105.0	216.4 ± 93.7	229.2 ± 107.0
rs16948048	*ZNF652*	28.1 ± 2.8	29.4 ± 5.9	28.9 ± 5.8	128.6 ± 73.7	124.8 ± 65.9	123.5 ± 66.1	215.0 ± 60.6	227.0 ± 96.3	226.5 ± 106.9

Abbreviations: BMI, body mass index; SFA, subcutaneous fat area; SNP, single nucleotide polymorphism; VFA, visceral fat area.
11, allele1/allele1; 12, allele1/allele2; 22, allele2/allele2. Allele 1 and allele 2 of each SNP is indicated in Table 2.

in the *CYP17A1* gene was significantly associated with reduced BMI (P=0.0018). The other SNPs were not significantly associated with BMI. No SNP was significantly associated with VFA. The A-allele of rs1004467 in the *CYP17A1* and the T-allele of rs11191548 in the *NT5C2* gene were significantly associated with reduced SFA. These SNPs are in linkage disequilibrium, as reported in the HapMap database (D'=0.98, r^2=0.71), and the A-allele of rs1004467 and T-allele of rs11191548 are reported to be risk alleles for increased blood pressure.[20,21]

BMI, VFA and SFA are known to be affected by gender; therefore, we compared rs1004467 and rs11191548 alleles with anthropometric parameters (BMI, VFA and SFA) in men and women independently (Table 5). Associations of both SNPs with VFA (P=0.0018 and P=0.0043) and SFA (P=0.00039 and P=0.0021) in women were significant, except the association of T-allele of rs11191548 with VFA. The VFA and SFA values of the rs11191548 genotype suggest that the recessive model would be the best-fitted model both in men and women. By using the recessive model, results revealed significant associations of the rs11191548 genotype with VFA (P=0.0022) and SFA in women (P=0.00059). These SNPs did not show any association with VFA or SFA in men, suggesting that they exhibit sexual dimorphism, as has been suggested in a recent report.[26] As both rs1004467 and rs11191548 were associated with a reduction in both VFA and SFA, we examined the association of these SNPs with total fat area. The SNPs were significantly associated with total fat area (P=0.00012 at rs1004467, P=0.00052 at rs11191548 in additive model) in women, but not in men, suggesting that risk allele for high blood pressure of these SNPs are associated with reduced adiposity in women. The very small mean F_{ST} value (0.00023) indicated no population structure in our subjects. As we collected the samples from nine institutes in four regions of Japan (Supplementary Table 1), we tested multiple linear regression analysis with age and institute as explanatory variables in men and women. Very similar results were observed. In additive model, significant associations of the

Table 4 Relationship between blood pressure-associated loci and adiposity measures

		BMI			*VFA*			*SFA*		
SNP ID	*Nearby gene*	β	*s.e.*	P-*value*	β	*s.e.*	P-*value*	β	*s.e.*	P-*value*
rs16998073	*FGF5*	−0.002	0.003	0.55	−0.003	0.010	0.78	−0.010	0.008	0.22
rs11014166	*CACNB2*	−0.005	0.007	0.48	−0.043	0.021	0.043	−0.008	0.017	0.63
rs1530440	*C10orf107*	−0.002	0.004	0.71	0.010	0.014	0.48	−0.005	0.011	0.64
rs1004467	*CYP17A1*	−0.010	0.003	**0.0018**	−0.022	0.010	0.027	−0.030	0.008	**0.00011**
rs11191548	*NT5C2*	−0.008	0.003	0.015	−0.019	0.011	0.078	−0.026	0.008	**0.0016**
rs381815	*PLEKHA7*	−0.007	0.004	0.046	−0.004	0.012	0.76	−0.015	0.009	0.10
rs2681472	*ATP2B1*	0.002	0.003	0.43	0.006	0.010	0.52	0.005	0.008	0.49
rs2681492	*ATP2B1*	0.003	0.003	0.34	0.006	0.010	0.54	0.006	0.008	0.40
rs6495112	*ARID3B*	−0.002	0.003	0.45	−0.004	0.010	0.65	−0.007	0.008	0.36
rs1378942	*CSK*	0.005	0.004	0.20	0.010	0.012	0.40	0.005	0.009	0.61
rs12946454	*PLCD3*	−0.003	0.004	0.39	0.009	0.013	0.50	−0.011	0.010	0.28
rs16948048	*ZNF652*	0.005	0.004	0.30	0.008	0.014	0.57	0.005	0.011	0.67

Abbreviations: BMI, body mass index; SFA, subcutaneous fat area; SNP, single nucleotide polymorphism; VFA, visceral fat area.
Data were derived from a linear regression analysis. The values of BMI, VFA and SFA were logarithmically transformed. Logarithmically transformed BMI, VFA and SFA were adjusted for age and gender. Tested alleles are risk alleles of increased blood pressure.

Table 5 Relationship between rs1004467 and rs11191548, and adiposity in men and women

			Values at each genotype			*Additive model*		*Recessive model*	
SNP ID (gene)	*Phenotype*	*Gender*	*11*	*12*	*22*	β *(s.e.)*	P-*value*	β *(s.e.)*	P-*value*
rs1004467	*n*	Men	233	259	64				
(*CYP17A1*)		Women	326	308	89				
	BMI ($kg\,m^{-2}$)	Men	29.7 ± 6.6	30.6 ± 5.9	30.1 ± 5.1	−0.011 (0.005)	0.029	−0.017 (0.006)	0.0085
		Women	27.6 ± 4.6	28.5 ± 6.0	28.9 ± 5.3	−0.010 (0.004)	0..017	−0.013 (0.006)	0.019
	VFA (cm^2)	Men	152.9 ± 67.7	160.6 ± 69.2	142.8 ± 59.9	0.004 (0.014)	0.78	−0.012 (0.019)	0.52
		Women	92.3 ± 49.2	105.4 ± 56.8	107.8 ± 54.7	−0.044 (0.014)	**0.0018**	−0.061 (0.019)	**0.0014**
	SFA (cm^2)	Men	198.6 ± 103.0	211.9 ± 113.8	215.4 ± 106.7	−0.028 (0.013)	0.037	−0.036 (0.018)	0.047
		Women	227.6 ± 82.7	248.0 ± 106.9	271.2 ± 103.3	−0.033 (0.009)	**0.00039**	−0.040 (0.013)	**0.0020**
rs11191548	*n*	Men	289	220	47				
(*NT5C2*)		Women	386	284	53				
	BMI ($kg\,m^{-2}$)	Men	30.0 ± 6.8	30.6 ± 5.4	29.4 ± 5.4	−0.007 (0.005)	0.19	−0.013 (0.006)	0.049
		Women	27.6 ± 4.7	28.7 ± 6.1	28.5 ± 4.8	−0.010 (0.004)	0.021	−0.015 (0.006)	0.0080
	VFA (cm^2)	Men	153.8 ± 68.0	161.3 ± 69.3	137.2 ± 54.8	0.007 (0.014)	0.65	−0.008 (0.018)	0.65
		Women	93.3 ± 49.3	107.4 ± 58.1	105.8 ± 52.8	−0.043 (0.015)	0.0043	−0.059 (0.019)	**0.0022**
	SFA (cm^2)	Men	202.1 ± 107.5	214.3 ± 111.3	199.9 ± 102.5	−0.023 (0.014)	0.10	−0.035 (0.018)	0.048
		Women	228.5 ± 84.9	257.4 ± 111.1	253.0 ± 89.0	−0.031 (0.010)	**0.0021**	−0.044 (0.013)	**0.00059**

Abbreviations: BMI, body mass index; SFA, subcutaneous fat area; SNP, single nucleotide polymorphism; VFA, visceral fat area.
Values are shown as the mean ± s.d. Data were derived from a linear regression analysis. The values of BMI, VFA and SFA were logarithmically transformed. Logarithmically transformed BMI, VFA and SFA were adjusted for age. Tested alleles (allele1 at both SNPs) are risk alleles of increased blood pressure.

rs1004467 and rs11191548 genotype with VFA (P=0.0015 and 0.0011, respectively) and SFA (P=0.00021 and 0.00062, respectively) were observed in women (Supplementary Table 2). Statistical analysis using analysis of covariance indicated significant associations of the rs1004467 and rs11191548 genotype with VFA (P=0.0020 and 0.0015, respectively) and SFA (P=0.00033 and 0.00042, respectively) in women (Supplementary Table 2). As some diabetes medications have an effect on adiposity,[27] we performed the analysis excluding 147 type 2 diabetic patients treated with sulfonylureas, biguanides and thiazolidinediones We found the similar significant associations of the rs1004467 and rs11191548 genotype with VFA and SFA in women (Supplementary Table 3).

We have reported that rs1558902 in the *FTO* gene is associated with both VFA and SFA,[11] and that rs7498665 in the *SH2B1* gene is associated with VFA.[17] Thus, we examined SNP×SNP epistasis in men, women and all subjects. The combination of rs1004467 and rs7498665 exhibited no epistatic effect on VFA in men (P=0.43), women (P=0.86) or all subjects (P=0.76). The combination of rs1004467 and rs1558902 did not show epistatic effect on VFA in men (P=0.99), women (P=0.53) or all subjects (P=0.60), or on SFA in men (P=0.63), women (P=0.83) or all subjects (P=0.89).

Among the SNPs tested in this study, rs16998073 in the *FGF5* gene and rs11191548 in the *NT5C2* gene were associated with increased systolic blood pressure ($P<0.05$). Rs11191548 in the *NT5C2* gene were also associated with hypertension ($P<0.05$). We could replicate the association between blood pressure and the above two SNPs that were reported to be strongly associated with blood pressure in the Japanese population (Supplementary Table 4).[28]

DISCUSSION

In this study, we showed that the A-allele of rs1004467 in the *CYP17A1* and the T-allele of rs11191548 in the *NT5C2* gene were significantly associated with reduced VFA, SFA and total fat area in women. Association of T-allele of rs11191548 in the *NT5C2* gene with increased systolic blood pressure and hypertension was replicated in our sample, as reported previously.[28] Our hypothesis was that these risk alleles would be associated with increased VFA and/or SFA as increased adiposity is a risk for hypertension;[4,5] however, these alleles affected decreased adiposity. The associations between SNPs and increased blood pressure/hypertension were evaluated after being adjusted for BMI, age and gender. Thus, the SNPs associated with visceral fat obesity-related and gender-dependent hypertension would be excluded in the screening stage. Indeed, recent analysis has shown that genetic variation near insulin receptor substrate 1 (*IRS1*) is associated with reduced adiposity and an impaired metabolic profile.[29] Thus, it is likely that rs1004467 and rs11191548 are associated with reduced VFA and SFA, as well as with hypertension in women.

The SNPs rs1004467 and rs11191548 were not associated with BMI in men or women, as reported for rs2943650 near *IRS1*.[29] As BMI represents both fat and lean body mass, our observation suggests that these SNPs influence a reduction in VFA and SFA, or influence an increased percentage of lean body mass. The significant associations of rs1004467 and rs1191548 with reduced VFA and SFA were observed in women, but not in men. The rs1004467 SNP is located in the intron of the *CYP17A1* gene. CYP17A1 is involved in the biosynthesis of glucocorticoids, mineral corticoids, androgens and estrogens.[30] The rs1004467 risk allele may reflect differences in *CYP17A1* gene expression that alter the biosynthesis of steroid hormones, leading to hypertension and reduced adiposity in women. The region of linkage disequilibrium that includes rs1004467 and rs11191548 contains a couple of genes in addition to *CYP17A1*: *NT5C2*, arsenic (+3 oxidation state) methyltransferase (*AS3MT*) and cyclin M2 (*CNNM2*). NT5C2 is a cytosolic IMP/GMP selective 5′-nucleotidase and involved in nucleic acids or DNA synthesis.[31] CNNM2 (ancient conserved domain protein, ACDP2) is a transporter of magnesium, which is required for the catalytic activity of numerous metalloenzymes.[32] Thus, these genes would be important for metabolism in adipocyte hyperplasia and hypertrophy. Further investigation is warranted to elucidate the functional SNPs and susceptibility genes.

We have previously reported that *FTO* rs1558902 is associated with VFA and SFA, and that *SH2B1* rs7498665 is associated with VFA.[11,17] Epistasis, or gene–gene interaction, has recently received much attention in human genetics.[33] In this study, the effect of these SNPs on VFA and SFA was additive, and an epistatic effect was not observed.

In summary, we showed that *CYP17A1* rs1004467 and *NT5C2* rs11191548 SNPs are significantly associated with both reduced VFA and SFA in women. Our results suggest that the region encompassing *CYP17A1* to *NT5C2* has a role in reducing visceral and subcutaneous fat mass. However, these results require confirmation in other populations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Carr, D. B., Utzschneider, K. M., Hull, R. L., Kodama, K., Retzlaff, B. M., Brunzell, J. D. *et al.* Intra-abdominal fat is a major determinant of the National Cholesterol Education Program Adult Treatment Panel III criteria for the metabolic syndrome. *Diabetes* **53,** 2087–2094 (2004).
2 Matsuzawa, Y. Therapy insight: adipocytokines in metabolic syndrome and related cardiovascular disease. *Nat. Clin. Pract. Cardiovasc. Med.* **3,** 35–42 (2006).
3 Hotta, K., Funahashi, T., Bodkin, N. L., Ortmeyer, H. K., Arita, Y., Hansen, B. C. *et al.* Circulating concentrations of the adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. *Diabetes* **50,** 1126–1133 (2001).
4 Mastuzawa, Y. Metabolic syndrome-definition and diagnostic criteria in Japan. *J. Atheroscler. Thromb.* **12,** 301 (2005).
5 Arai, H., Yamamoto, A., Matsuzawa, Y., Saito, Y., Yamada, N., Oikawa, S. *et al.* Prevalence of metabolic syndrome in the general Japanese population in 2000. *J. Atheroscler. Thromb.* **13,** 202–208 (2006).
6 Selby, J. V., Newman, B., Quesenberry, C. P. Jr., Fabsitz, R. R., Carmelli, D., Meaney, F. J. *et al.* Genetic and behavioral influences on body fat distribution. *Int. J. Obes.* **14,** 593–602 (1990).
7 Rose, K. M., Newman, B., Mayer-Davis, E. J. & Selby, J. V. Genetic and behavioral determinants of waist-hip ratio and waist circumference in women twins. *Obes. Res.* **6,** 383–392 (1998).
8 Souren, N. Y., Paulussen, A. D., Loos, R. J., Gielen, M., Beunen, G., Fagard, R. *et al.* Anthropometry, carbohydrate and lipid metabolism in the East Flanders Prospective Twin Survey: heritabilities. *Diabetologia* **50,** 2107–2116 (2007).
9 Lindgren, C. M., Heid, I. M., Randall, J. C., Lamina, C., Steinthorsdottir, V., Qi, L. *et al.* Genome-wide association scan meta-analysis identifies three loci influencing adiposity and fat distribution. *PLoS Genet.* **5,** e1000508 (2009).
10 Heard-Costa, N. L., Zillikens, M. C., Monda, K. L., Johansson, A., Harris, T. B., Fu, M. *et al.* NRXN3 is a novel locus for waist circumference: a genome-wide association study from the CHARGE Consortium. *PLoS Genet.* **5,** e1000539 (2009).
11 Hotta, K., Nakamura, M., Nakamura, T., Matsuo, T., Nakata, Y., Kamohara, S. *et al.* Polymorphisms in NRXN3, TFAP2B, MSRA, LYPLAL1, FTO and MC4R and their effect on visceral fat area in the Japanese population. *J. Hum. Genet.* **55,** 738–742 (2010).
12 Tanabe, A., Yanagiya, T., Iida, A., Saito, S., Sekine, A., Takahashi, A. *et al.* Functional single-nucleotide polymorphisms in the secretogranin III (SCG3) gene that form secretory granules with appetite-related neuropeptides are associated with obesity. *J. Clin. Endocrinol. Metab.* **92,** 1145–1154 (2007).
13 Yanagiya, T., Tanabe, A., Iida, A., Saito, S., Sekine, A., Takahashi, A. *et al.* Association of single-nucleotide polymorphisms in MTMR9 gene with obesity. *Hum. Mol. Genet.* **16,** 3017–3026 (2007).
14 Thorleifsson, G., Walters, G. B., Gudbjartsson, D. F., Steinthorsdottir, V., Sulem, P., Helgadottir, A. *et al.* Genome-wide association yields new sequence variants at seven loci that associate with measures of obesity. *Nat. Genet.* **41,** 18–24 (2009).
15 Willer, C. J., Speliotes, E. K., Loos, R. J., Li, S., Lindgren, C. M., Heid, I. M. *et al.* Six new loci associated with body mass index highlight a neuronal influence on body weight regulation. *Nat. Genet.* **41,** 25–34 (2009).
16 Meyre, D., Delplanque, J., Chèvre, J. C., Lecoeur, C., Lobbens, S., Gallina, S. *et al.* Genome-wide association study for early-onset and morbid adult obesity identifies three new risk loci in European populations. *Nat. Genet.* **41,** 157–159 (2009).
17 Hotta, K., Kitamoto, T., Kitamoto, A., Mizusawa, S., Matsuo, T., Nakata, Y. *et al.* Computed tomography analysis of the association between SH2B1 rs7498665 single-nucleotide polymorphism and visceral fat area. *J. Hum. Genet.* **56,** 716–719 (2011).
18 Hindorff, L. A., Sethupathy, P., Junkins, H. A., Ramos, E. M., Mehta, J. P., Collins, F. S. *et al.* Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. *Proc. Natl Acad. Sci. USA* **106,** 9362–9367 (2009).
19 Hotta, K., Kitamoto, T., Kitamoto, A., Mizusawa, S., Matsuo, T., Nakata, Y. *et al.* Association of variations in the FTO, SCG3 and MTMR9 genes with metabolic syndrome in a Japanese population. *J. Hum. Genet.* **56,** 647–651 (2011).
20 Levy, D., Ehret, G. B., Rice, K., Verwoert, G. C., Launer, L. J., Dehghan, A. *et al.* Genome-wide association study of blood pressure and hypertension. *Nat. Genet.* **41,** 677–687 (2009).
21 Newton-Cheh, C., Johnson, T., Gateva, V., Tobin, M. D., Bochud, M., Coin, L. *et al.* Genome-wide association study identifies eight loci associated with blood pressure. *Nat. Genet.* **41,** 666–676 (2009).
22 Yoshizumi, T., Nakamura, T., Yamane, M., Islam, A. H., Menju, M., Yamasaki, K. *et al.* Abdominal fat: standardized technique for measurement at CT. *Radiology* **211,** 283–286 (1999).
23 Ohnishi, Y., Tanaka, T., Ozaki, K., Yamada, R., Suzuki, H. & Nakamura, Y. A high-throughput SNP typing system for genome-wide association studies. *J. Hum. Genet.* **46,** 471–477 (2001).
24 Nielsen, D. M., Ehm, M. G. & Weir, B. S. Detecting marker-disease association by testing for Hardy–Weinberg disequilibrium at a marker locus. *Am. J. Hum. Genet.* **63,** 1531–1540 (1998).
25 Wright, S. The genetical structure of populations. *Ann. Eugen.* **15,** 323–354 (1951).
26 Heid, I. M., Jackson, A. U., Randall, J. C., Winkler, T. W., Qi, L., Steinthorsdottir, V. *et al.* Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. *Nat. Genet.* **42,** 949–960 (2010).

27 Mitri, J. & Hamdy, O. Diabetes medications and body weight. *Expert Opin. Drug Saf.* **8,** 573–584 (2009).
28 Takeuchi, F., Isono, M., Katsuya, T., Yamamoto, K., Yokota, M., Sugiyama, T. *et al.* Blood pressure and hypertension are associated with 7 loci in the Japanese population. *Circulation* **121,** 2302–2309 (2010).
29 Kilpeläinen, T. O., Zillikens, M. C., Stančákova, A., Finucane, F. M., Ried, J. S., Langenberg, C. *et al.* Genetic variation near IRS1 associates with reduced adiposity and an impaired metabolic profile. *Nat. Genet.* **43,** 753–760 (2011).
30 Gilep, A. A., Sushko, T. A. & Usanov, S. A. At the crossroads of steroid hormone biosynthesis: the role, substrate specificity and evolutionary development of CYP17. *Biochim. Biophys. Acta* **1814,** 200–209 (2011).
31 Itoh, R. IMP–GMP 5′-nucleotidase. *Comp. Biochem. Physiol. B* **105,** 13–19 (1993).
32 Goytain, A. & Quamme, G. A. Functional characterization of ACDP2 (ancient conserved domain protein), a divalent metal transporter. *Physiol. Genomics* **22,** 382–389 (2005).
33 Cordell, H. Detecting gene–gene interactions that underlie human diseases. *Nat. Rev. Genet.* **10,** 392–404 (2009).

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Class II HLA genotype in fulminant type 1 diabetes: A nationwide survey with reference to glutamic acid decarboxylase antibodies

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ABSTRACT

Aims/Introduction: Fulminant type 1 diabetes is a subtype of type 1 diabetes characterized by a remarkably abrupt onset of insulin-deficient hyperglycemia within a few days. The aim of the present study was to clarify characteristic class II HLA genotypes in a large number of patients with fulminant type 1 diabetes to date.

Materials and Methods: We analyzed the HLA-*DRB1* and *DQB1* genotypes, and their haplotypes in 207 patients with fulminant type 1 diabetes and 325 control subjects in the Japanese population.

Results: The frequencies of the *DRB1*04:05-DQB1*04:01* and *DRB1*09:01-DQB1*03:03* haplotypes were significantly higher, and those of the *DRB1*01:01-DQB1*05:01*, *DRB1*15:02-DQB1*06:01* and *DRB1*08:03-DQB1*06:01* haplotypes were significantly lower in patients with fulminant type 1 diabetes than in the control subjects. Combination analysis showed that the frequencies of homozygotes with *DRB1*04:05-DQB1*04:01* [odds ratio (OR) 7.0] and *DRB1*09:01-DQB1*03:03* (OR 9.5) were significantly higher in patients with fulminant type 1 diabetes. Within a limited portion of patients with fulminant type 1 diabetes with antibodies to glutamic acid decarboxylase (GADab; $n = 25$), the frequency of *DRB1*09:01-DQB1*03:03*, but not *DRB1*04:05-DQB1*04:01*, was significantly higher than in control subjects (44.0% vs 13.7%; $Pc < 0.05$, OR 5.0).

[Correction to last line of Results, added after online publication 29 July 2011: "OR 5.1" is changed to "OR 5.0".]

Conclusions: Our large-scale study showed the characteristic class II HLA genotypes in fulminant type 1 diabetes, and implicated that genetic contribution to disease susceptibility is distinct between GADab-positive and GADab-negative fulminant type 1 diabetes. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2011.00139.x, 2012)

KEY WORDS: Fulminant type 1 diabetes, HLA, Glutamic acid decarboxylase

INTRODUCTION

Fulminant type 1 diabetes is a novel subtype of type 1 diabetes identified in 2000[1–3]. It is defined as diabetes that results from the extremely rapid and almost entire destruction of pancreatic β-cells within a few days. The clinical characteristics of this subtype are different in many aspects from those of typical type 1A diabetes[3]. Although fulminant type 1 diabetes resembles the typical form of type 1 diabetes in that it is characterized by high plasma glucose levels accompanied by ketosis or ketoacidosis, it clearly differs by an extremely acute onset of diabetes, which is confirmed by nearly normal HbA_{1c} levels against high plasma glucose concentration, and virtually no C-peptide secretion at the onset of the disease, indicating that the process of pancreatic β-cell destruction is very rapid.

Fulminant type 1 diabetes is common in the Asian population; it accounts for approximately 20% of ketosis-onset type 1 diabetes in Japan[2,3] and 7% in Korea[4,5]. Furthermore, several cases have been reported from China[6], Taiwan[7], the Philippines[8], Malaysia[9] and France[10].

It is suggested that both genetic factors[11–13] and environmental factors, such as viral infection[14–19], contribute to the pathogenesis of this disease. In regard to genetic factors, it has been reported that class II HLA strongly confers susceptibility to the development of fulminant type 1 diabetes. In the analysis of the serological typing of class II HLA, we have shown that HLA-DR4-DQ4 was significantly more frequent in fulminant type 1 diabetes in Japan[12]. Several studies have so far reported the association of class II HLA genotype with fulminant type 1 diabetes[20–22]; however, the number of patients was limited in these reports as a result of the low incidence of type 1 diabetes in general, fulminant type 1 diabetes in particular, in the Japanese population.

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The aim of the present study was thus to investigate the class II HLA genotypes and re-evaluate the contribution of the class II HLA to susceptibility and resistance to fulminant type 1 diabetes in a large number of patients.

MATERIALS AND METHODS

Subjects and Methods

We examined 207 patients with fulminant type 1 diabetes and 325 healthy control subjects in Japan. Among them, 152 patients with fulminant type 1 diabetes were registered with the committee of the Japan Diabetes Society, and data for the other 55 patients were collected from reports in the literature from June 2000 to March 2007.

Inclusion criteria for fulminant type 1 diabetes were: (i) ketosis or ketoacidosis within a week after the onset of hyperglycemic symptoms; (ii) urinary C-peptide excretion <10 μg/day or fasting serum C-peptide <0.3 ng/mL (0.10 nmol/L) or serum C-peptide <0.5 ng/mL (0.17 nmol/L) after glucagon injection or meal load soon after disease onset; and (iii) plasma glucose level ≥16.0 mmol/L (288 mg/dL) and HbA_{1c} <8.9% at the first visit[2]. Healthy control subjects had normal glucose tolerance as assessed by a 75 g oral glucose tolerance test, had no family history of diabetes, and resided in the Ehime and Osaka areas as described previously[23]. GAD antibodies (GADab) were positive in 25 patients and negative in 182 patients (Table 1). We also analyzed 15 patients with pregnancy-associated fulminant type 1 diabetes (PF), 51 female patients of child-bearing age (13–49 years) with fulminant type 1 diabetes that was not associated with pregnancy (NPF) and 70 female control subjects of child-bearing age.

The present study was approved by the ethics committee of the Japan Diabetes Society, and informed consent was obtained from all subjects. The detailed characteristics of these subjects are shown in Table 1.

The value for HbA_{1c} (%) was estimated as a National Glycohemoglobin Standardization Program (NGSP) equivalent value (%) calculated by the formula HbA_{1c} (%) = HbA_{1c} (JDS) (%) + 0.4%, considering the relational expression of HbA_{1c} (JDS) (%) measured by the previous Japanese standard substance and measurement methods and HbA_{1c} (NGSP)[24].

Typing of HLA-DR and -DQ

HLA-DRB1 and *-DQB1* were genotyped by the PCR sequence-specific primer and PCR sequence-specific oligonucleotide methods (Invitrogen, Carlsbad, CA, USA). The most probable *DRB1-DQB1* haplotypes were deduced from known linkage disequilibria.

Statistical Analysis

Clinical data of GADab-negative and -positive fulminant type 1 diabetes was analyzed by using chi-squared-test or Kruskal–Wallis test. Allele frequencies were estimated by direct counting. Genotypes, whose total frequencies in both total subjects with fulminant type 1 diabetes and control subjects were five or more than five, were listed in the present study. The significance of the difference in distribution of alleles between patients with fulminant type 1 diabetes and healthy control subjects was determined by a chi-squared-test. *P*-values were corrected by using the number of different alleles tested (denoted as *Pc*). Statistical significance was defined as $Pc < 0.05$.

RESULTS

Characteristics of GADab-Negative and -Positive Fulminant Type 1 Diabetes

GADab was detected in 25 (12.1%) of 207 patients with fulminant type 1 diabetes in the present study. Therefore, first of all, we compared detailed characteristics between GADab-negative and -positive fulminant type 1 diabetes (Table 1). There were no differences between the two groups in age, body mass index, mean HbA_{1c} level at onset and presence or absence of family history of type 1 or type 2 diabetes in first-degree relatives. One, but not another, allele of class II HLA haplotype was common between two patients (father and his son) with a family history of

Table 1 | Clinical characteristics of patients with fulminant type 1 diabetes

	Total	With GADab	Without GADab	Control
n	207	25 (12.1)	182 (87.9)	325
Sex (male/female)	118/89 (57.0)	20/5 (80.0)	98/84 (53.8)	202/123 (62.2)
Pregnancy (PF*/NPF†)	15/51 (22.7)	0/5 (0.0)	15/49 (23.4)	ND
Age at disease onset (years)	41 (0–87)	43 (0–75)	41 (1–87)	47 (25–78)
Body mass index (kg/m^2)	21.1 ± 3.2‡	20.9 ± 3.4§	21.2 ± 3.2¶	ND
Family history of type 1 diabetes	5/157 (3.1)	0/20 (0.0)	5/137 (3.5)	0/0 (0.0)
Family history of type 2 diabetes	11/151 (6.8)	2/18 (10.0)	9/133 (6.3)	0/0 (0.0)
Family history of unclassified diabetes	6/156 (3.7)	1/19 (5.0)	5/137 (3.5)	0/0 (0.0)
HbA_{1c} at disease onset (%)	6.6 ± 0.8	6.7 ± 0.7	6.6 ± 0.8	ND

GADab, antibodies to glutamic acid decarboxylase; ND, not determined.
Data are *n*, median (range), mean ± SD, (±), or *n* (%).
*Pregnancy-associated fulminant type 1 diabetes; †Female patients of child-bearing age (13–49 years) with fulminant type 1 diabetes not associated with pregnancy; ‡except seven children; §Except two children; ¶Except five children.