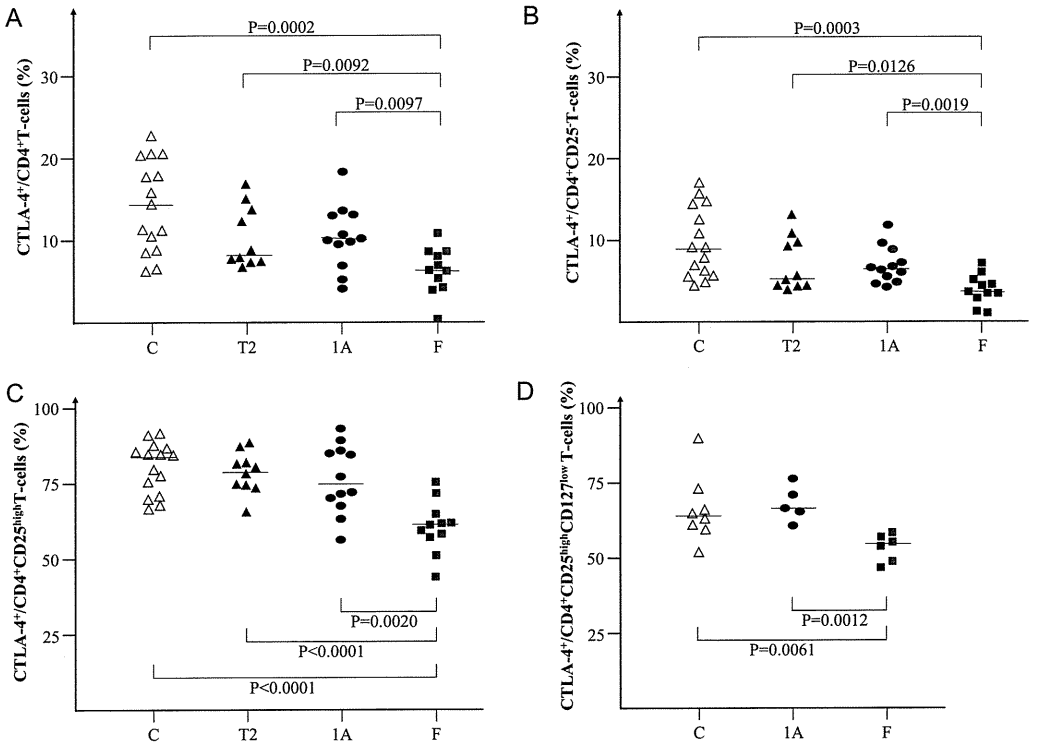

Fig. 2. Expression of intracellular CTLA-4. Reduced expression of intracellular CTLA-4 in $CD4^+$ helper T-cells (A), $CD4^+$ $CD25^-$ T-cells (B), $CD4^+CD25^{high}$ T-cells (C) and $CD4^+CD25^{high}CD127^{low}$ T-cells (D) from patients with fulminant type 1 diabetes. Data plotted represent the intracellular CTLA-4 level in each T-cell subset from normal healthy control subjects (C, open triangles), from patients with type 2 diabetes (T2, closed triangles), from patients with type 1 diabetes (1A, closed circles) and from patients with fulminant type 1 diabetes (F, closed squares). Bars represent the median.

type 1 diabetes when compared with normal control subjects, and patients with type 1A and type 2 diabetes. Reduced CTLA-4 expression was also confirmed in both $CD4^+CD25^{high}$ T-cells and $CD4^+CD25^-$ T-cells. These facts indicated that CTLA-4 would be a good diagnostic marker for fulminant type 1 diabetes. Furthermore, the reduced expression of intracellular CTLA-4 could explain the reason of the explosive immune reactivity and subsequent destruction of pancreatic beta cells observed during the clinical course of fulminant type 1 diabetes. The lack of CTLA-4 might lead to dysregulated activation of effector T-cells independently of Tregs. As far as we know, this is the first report examining intracellular CTLA-4 expression in either type 1 or type 2 diabetes that demonstrated reduced expression of the molecule in fulminant type 1 diabetes.

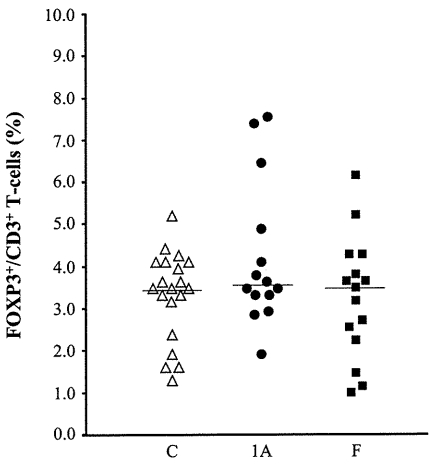

Fig. 3. Frequency of $FOXP3^+$ T-cells among $CD3^+$ T-cells. Frequency of $FOXP3^+$ T-cells (%) among $CD3^+$ T-cells from normal healthy control subjects (C, open triangles), from patients with type 1 diabetes (1A, closed circles) and from patients with fulminant type 1 diabetes (F, closed squares). Bars represent the median.

We have shown a significant reduction in CTLA-4 expression in $CD4^+CD25^-$ T-cells from patients with fulminant type 1 diabetes. There was a significant negative correlation between the proliferation of $CD4^+CD25^-$ T-cells and their levels of CTLA-4. The importance of CTLA-4 as an inhibitor of T-cell activation was clearly demonstrated using CTLA-4 knockout mice, which mice develop massive and rapidly fatal T-cell lymphoproliferation [20]. In other CTLA-4 deficient mice, irregular immune reactions were observed and beta cells were rapidly destroyed after the adoptive transfer of T cells that recognized beta-cell antigens [21]. These findings suggest that reduced expression of intracellular CTLA-4 in $CD4^+CD25^-$ T-cells might lead to an increase in the proliferation of $CD4^+CD25^-$ T-cells and result in the markedly rapid destruction of pancreatic beta cells, as seen during the clinical course of fulminant type 1 diabetes.

We have also shown a significant reduction in CTLA-4 expression in $CD4^+CD25^{high}$ T-cells from patients with fulminant type 1 diabetes. No significant correlation was observed between proliferation and the levels of CTLA-4 in $CD4^+CD25^{high}$ T-cells. This may be because $CD4^+CD25^{high}$ T-cells are themselves anergic, that is, they are unresponsive to TCR stimulation. In addition, there was no significant difference in either the frequency of $CD3^+FOXP3^+$ T-cells or the suppressive function of $CD4^+CD25^{high}$ T-cells among the three groups. An increased rate of CTLA-4 internalization may contribute to abnormal Treg function as shown in rheumatoid arthritis [22]. Further investigation is necessary to characterize the pathway leading from the reduced expression of CTLA-4 in $CD4^+CD25^{high}$ T-

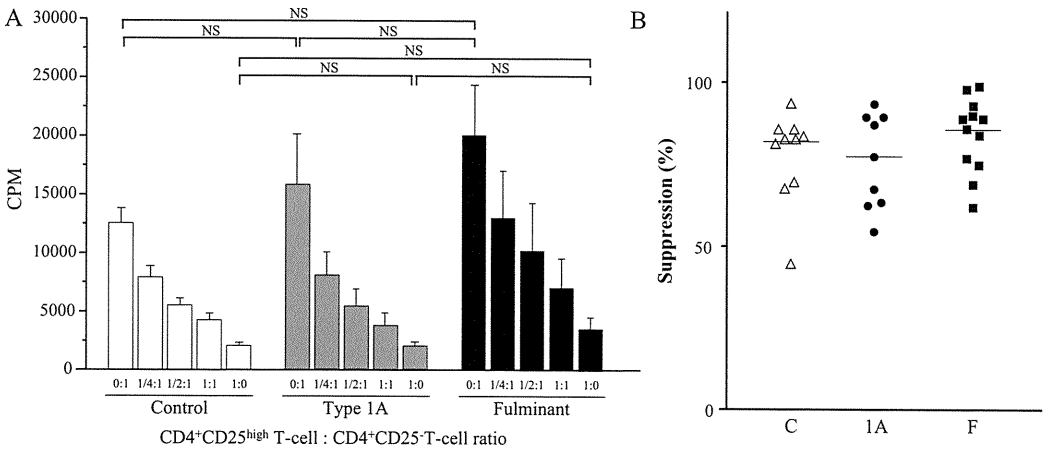

Fig. 4. Function of $CD4^+CD25^-$/$CD4^+CD25^{high}$ T-cells. (A) Proliferation in separated cell populations and coculture. Representative proliferation ($[^3H]$thymidine incorporation) of 1×10^4 $CD4^+CD25^-$ T-cells from normal healthy control subjects (open bars), from patients with type 1A diabetes (gray bars) and from patients with fulminant type 1 diabetes (closed bars) in the presence of different ratios of $CD4^+CD25^{high}$ T-cells. Bars represent the mean ±SE. (B) Function of $CD4^+CD25^{high}$ T-cells assessed by their ability to suppress the proliferation of $CD4^+CD2^-$ T-cells. Suppressive function of $CD4^+CD25^{high}$ T-cells co-cultured with $CD4^+CD25^-$ T-cells at a 1:1 ratio. Data plotted represent the percentage suppression of proliferation by $CD4^+CD25^{high}$ T-cells from normal healthy control subjects (C, open triangles), from patients with type 1 diabetes (1A, closed circles) and from patients with fulminant type 1 diabetes (F, closed squares). Bars represent the median.

cells to the accelerated immune reactivity of $CD4^+CD25^-$ T-cells in fulminant type 1 diabetes.

The mechanism behind the reduced expression of intracellular CTLA-4 was not clarified in this study. We have previously shown that the *CT60AA* subtype of *CTLA-4* was more prevalent and a lower level of soluble CTLA-4 was also found in patients with fulminant type 1 diabetes [19]. However, there was no correlation between the reduced CTLA-4 expression and either of the polymorphisms in the *CTLA-4* gene tested in this study. A low level of soluble CTLA-4 concentration in sera in our previous study [19] corresponded to reduced expression of intracellular CTLA-4 in fulminant type 1 diabetes in our present study. However, no such reduction in CTLA-4 mRNA expression was detected. Based on these findings, the reduced expression of intracellular CTLA-4 might be due to mechanisms that do not affect transcription.

In conclusion, the expression of intracellular CTLA-4 was clearly reduced in $CD4^+$ helper T-cells from patients with fulminant type 1 diabetes even after the onset of the disease. Reduced expression of CTLA-4 in $CD4^+$ helper T-cells might promote an uncontrollable immune reaction that then leads to accelerated beta cell loss and the development of fulminant type 1 diabetes.

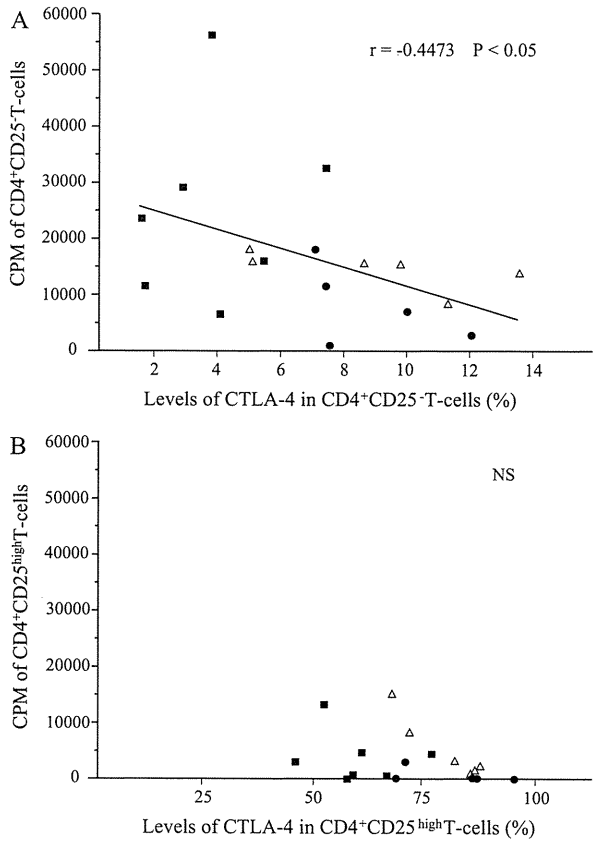

Fig. 5. Correlation between the proliferation and the levels of CTLA-4. Data plotted the correlation between the proliferation and the levels of CTLA-4 in $CD4^+CD25^-$ T-cells (A) and $CD4^+CD25^{high}$ T-cells (B) from normal healthy control subjects (open triangles), from patients with type 1 diabetes (closed circles) and from patients with fulminant type 1 diabetes (closed squares).

Conflict of interest

None.

Acknowledgements

We would like to thank Professor Yasuichiro Nishimura Dr Tetsuya Hiraiwa for his advice regarding to statistical analysis and Shinobu Mitsui and Teruo Ueno for their excellent technical assistance. This study was supported in part by a Grant-in Aid from the Japanese Society for the Promotion of Science (KAKENHI 19790641, 19591087, 19591069), a grant from The Naito Foundation (2007) a grant from the Takeda Science Foundation (2007) and a Health and Labour Sciences Research Grant on Research on Intractable Diseases from the Japanese Ministry of Health, Labour and Welfare.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.imlet.2011.05.003.

References

[1] Imagawa A, Hanafusa T, Miyagawa J, Matsuzawa Y. A novel subtype of type 1 diabetes mellitus characterized by a rapid onset and an absence of diabetes-related antibodies Osaka IDDM Study Group. N Engl J Med 2000;342:301–7.

[2] Imagawa A, Hanafusa T, Uchigata Y, Kanatsuka A, Kawasaki E, Kobayashi T, et al. Fulminant type 1 diabetes: a nationwide survey in Japan. Diabetes Care 2003;26:2345–52.

[3] Hanafusa T, Imagawa A. Fulminant type 1 diabetes: a novel clinical entity requiring special attention by all medical practitioners. Nat Clin Pract Endocrinol Metab 2007;3:36–45.

[4] Imagawa A, Hanafusa T, Uchigata Y, Kanatsuka A, Kawasaki E, Kobayashi T, et al. Different contribution of class II HLA in fulminant and typical autoimmune type 1 diabetes mellitus. Diabetologia 2005;48:294–300 [Erratum in: Diabetologia, 51, 524–526, 2008].

[5] Tanaka S, Kobayashi T, Momotsu T. A novel subtype of type 1 diabetes mellitus. N Engl J Med 2000;342:1835–7.

[6] Shibasaki S, Imagawa A, Tauriainen S, Iino M, Oikarinen M, Abiru H, et al. Expression of toll-like receptors in pancreas of recent-onset fulminant type 1 diabetes. Endocrinol J 2010;57:211–9.

[7] Kotani R, Nagata M, Imagawa A, Moriyama H, Yasuda H, Miyagawa J, et al. T lymphocyte response against pancreatic beta cell antigens in fulminant type 1 diabetes. Diabetologia 2004;47:1285–91.

[8] Linsley PS, Bradshaw J, Greene J, Peach R, Bennett KL, Mittler RS. Intracellular trafficking of CTLA-4 and focal localization towards sites of TCR engagement. Immunity 1996;4:535–43.

[9] Egen JG, Allison JP. Cytotoxic T lymphocyte antigen-4 accumulation in the immunological synapse is regulated by TCR signal strength. Immunity 2002;16:23–35.

[10] Rudd CE. The reverse stop-signal model for CTLA4 function. Nat Rev Immunol 2008;8:153–60.

[11] Ise W, Kohyama M, Nutsch KM, Lee HM, Suri A, Unanue ER, et al. CTLA-4 suppresses the pathogenicity of self antigen-specific T cells by cell-intrinsic and cell-extrinsic mechanism. Nat Immunol 2010;11:129–35.

[12] Sakaguchi S, Ono M, Setoguchi R, Yagi H, Hori S, Fehervari Z, et al. Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease. Immunol Rev 2006;212:8–27.

[13] Wildin RS, Ramsdell F, Peake J, Faravelli F, Casanova JL, Buist N, et al. X-linked neonatal diabetes mellitus, enteropathy and endocrinopathy syndrome is the human equivalent of mouse scurfy. Nat Genet 2001;27:18–20.

[14] Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko SA, et al. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet 2001;27:68–73.

[15] Bennett CL, Christie J, Ramsdell F, Brunkow ME, Ferguson PJ, Whitesell L, et al. The immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome (IPEX) is caused by mutations of FOXP3. Nat Genet 2001;27:20–1.

[16] Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T, Miyara M, Fehervari Z, et al. CTLA-4 control over Foxp3+ regulatory T cell function. Science 2008;322:271–5.

[17] Seino Y, Nanjo K, Tajima N, Kadowaki T, Kashiwagi A, Akai E, et al. The Committee of Japan Diabetes Society on the diagnostic criteria of diabetes mellitus; Report of the Committee on the classification and diagnostic criteria of diabetes mellitus. J Diabetes Invest 2010;1:212–28.

[18] Ueda H, Howson JMM, Esposito L, Heward J, Snook J, Chmberlain G, et al. Association of the T-cell regulatory gene CTLA-4 with susceptibility to autoimmune disease. Nature 2003;423:506–11.

[19] Kawasaki E, Imagawa A, Makino H, Uga M, Abiru N, Hanafusa T, et al. Differences in the contribution of the CTLA4 gene to susceptibility to fulminant and type 1A diabetes in Japanese patients. Diabetes Care 2008;31:1608–10.

[20] Tivol EA, Borriello F, Schweitzer AN, Lynch WP, Bluestone JA, Sharp AH. Loss of CTLA-4 leads to massive lymphoproliferation and fetal multiorgan tissue destruction, revealing a critical negative regulatory role of CTLA-4. Immunology 1995;3:541–7.

[21] Schmidt EM, Wang CJ, Ryan GA, Clough LE, Qureshi OS, Goodall M, et al. Ctla-4 controls regulatory T cell peripheral homeostasis and required for suppression of pancreatic islet autoimmunity. J Immunol 2009;182:274–82.

[22] Flores-Borja F, Jury EC, Mauri C, Ehrenstein MR. Defects in CTLA-4 are associated with abnormal regulatory T cell function in rheumatoid arthritis. Proc Natl Acad Sci U S A 2008;105:19396–21940.

Journal of Human Genetics (2011) 56, 716–719

www.nature.com/jhg

ORIGINAL ARTICLE

Computed tomography analysis of the association between the *SH2B1* rs7498665 single-nucleotide polymorphism and visceral fat area

Kikuko Hotta[1], Takuya Kitamoto[1], Aya Kitamoto[1], Seiho Mizusawa[2], Tomoaki Matsuo[3], Yoshio Nakata[3], Hideyuki Hyogo[4], Hidenori Ochi[4], Seika Kamohara[5], Nobuyuki Miyatake[6], Kazuaki Kotani[7], Ryoya Komatsu[8], Naoto Itoh[9], Ikuo Mineo[10], Jun Wada[11], Masato Yoneda[12], Atsushi Nakajima[12], Tohru Funahashi[13], Shigeru Miyazaki[14], Katsuto Tokunaga[15], Hiroaki Masuzaki[16], Takato Ueno[17], Kazuaki Chayama[4], Kazuyuki Hamaguchi[18], Kentaro Yamada[19], Toshiaki Hanafusa[20], Shinichi Oikawa[21], Hironobu Yoshimatsu[22], Toshiie Sakata[22], Kiyoji Tanaka[3], Yuji Matsuzawa[7], Kazuwa Nakao[1,23] and Akihiro Sekine[1,2]

Visceral fat accumulation has an important role in increasing morbidity and mortality rate by increasing the risk of developing several metabolic disorders, such as type 2 diabetes, dyslipidemia and hypertension. New genetic loci that contribute to the development of obesity have been identified by genome-wide association studies in Caucasian populations. We genotyped 1279 Japanese subjects (556 men and 723 women), who underwent computed tomography (CT) for measuring visceral fat area (VFA) and subcutaneous fat area (SFA), for the following single-nucleotide polymorphisms (SNPs): *NEGR1* rs2815752, *SEC16B* rs10913469, *TMEM18* rs6548238, *ETV5* rs7647305, *GNPDA2* rs10938397, *BDNF* rs6265 and rs925946, *MTCH2* rs10838738, *SH2B1* rs7498665, *MAF* rs1424233, and *KCTD15* rs29941 and rs11084753. In the additive model, none of the SNPs were significantly associated with body mass index (BMI). The *SH2B1* rs7498665 risk allele was found to be significantly associated with VFA (P=0.00047) but not with BMI or SFA. When the analysis was performed in men and women separately, no significant associations with VFA were observed (P=0.0099 in men and P=0.022 in women). None of the other SNPs were significantly associated with SFA. Our results suggest that there is a VFA-specific genetic factor and that a polymorphism in the *SH2B1* gene influences the risk of visceral fat accumulation.
Journal of Human Genetics (2011) **56**, 716–719; doi:10.1038/jhg.2011.86; published online 28 July 2011

Keywords: computed tomography; Japanese subjects; obesity; *SH2B1*; visceral fat area

INTRODUCTION

Obesity, especially visceral fat obesity, is a risk factor for several metabolic disorders, including type 2 diabetes, dyslipidemia and hypertension.[1] Several studies have indicated that adipose tissue, especially that in the visceral region, secretes various adipocytokines and that an increase in adipose tissue mass leads to alteration 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 in terms of waist circumference; waist-hip ratio; or visceral fat area (VFA), which is measured using computed tomography (CT).[1,4,5] Recently, two genome-wide association studies were conducted to identify the loci

[1]EBM Research Center, Kyoto University Graduate School of Medicine, Kyoto, Japan; [2]Center for Genomic Medicine, Unit of Genome Informatics, Kyoto University Graduate School of Medicine, Kyoto, Japan; [3]Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Japan; [4]Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan; [5]Health Science University, Yamanashi, Japan; [6]Department of Hygiene, Faculty of Medicine, Kagawa University, Kagawa, Japan; [7]Department of Metabolic Medicine, Graduate School of Medicine, Osaka University, Osaka, Japan; [8]Rinku General Medical Center, Osaka, Japan; [9]Toyonaka Municipal Hospital, Osaka, Japan; [10]Otemae Hospital, Osaka, Japan; [11]Department of Medicine and Clinical Science, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama, Japan; [12]Division of Gastroenterology, Yokohama City University Graduate School of Medicine, Yokohama, Japan; [13]Department of Metabolism and Atherosclerosis, Graduate School of Medicine, Osaka University, Osaka, Japan; [14]Tokyo Postal Services Agency Hospital, Tokyo, Japan; [15]Itami City Hospital, Hyogo, Japan; [16]Division of Endocrinology and Metabolism, Second Department of Internal Medicine, University of the Ryukyus Faculty of Medicine, Okinawa, Japan; [17]Research Center for Innovative Cancer Therapy, Kurume University, Kurume, Japan; [18]Department of Community Health and Gerontological Nursing, Faculty of Medicine, Oita University, Oita, Japan; [19]Division of Endocrinology and Metabolism, Department of Medicine, Kurume University, Kurume, Japan; [20]Department of Internal Medicine (I), Osaka Medical College, Osaka, Japan; [21]Division of Endocrinology and Metabolism, Department of Medicine, Nippon Medical School, Tokyo, Japan; [22]Department of Internal Medicine 1, Faculty of Medicine, Oita University, Oita, Japan and [23]Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan
Correspondence: Dr K Hotta, Assistant Professor, EBM Research Center, Kyoto University Graduate School of Medicine, Yoshida-Konoecho, Sakyo-ku, Kyoto 606-8501, Japan.
E-mail: kikukoh@kuhp.kyoto-u.ac.jp
Received 6 June 2011; revised 29 June 2011; accepted 30 June 2011; published online 28 July 2011

linked with waist circumference and waist-hip ratio.[6,7] In a previous study, we have 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 subcutaneous fat area (SFA) and body mass index (BMI).[8]

We performed a large-scale, case–control association study and found that secretogranin III (*SCG3*)[9] and myotubularin-related protein 9 (*MTMR9*)[10] conferred susceptibility to an obese phenotype in the Japanese population. Recent progress in genome-wide association studies has increased the number of known genetic susceptibility loci for obesity.[11–13] Some of the obesity-associated loci identified by the genome-wide association studies were found to be replicated in the Japanese population.[14,15] Some of the obesity-related loci were found to overlap with the waist circumference waist-hip ratio-related loci, for example, the loci within the *FTO* gene and near the melanocortin 4 receptor (*MC4R*) gene.

In this study, we investigated whether the recently reported obesity-related loci were associated with VFA, which is an important factor responsible for increased morbidity and mortality rates.

MATERIALS AND METHODS

Study subjects

In this study, we enrolled 1279 Japanese subjects from outpatient clinics; these patients had agreed to undergo CT testing (in the supine position) to determine the VFA and SFA values at the umbilical level (L4–L5). Both VFA and SFA values were calculated using the FatScan software program (N2system, Osaka, Japan).[16] The patients visited the hospitals to undergo the 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 or under treatment that strongly affect the body weight were also excluded. The clinical data were taken 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 single-nucleotide polymorphism genotyping

Genomic DNA was extracted from the blood samples collected from each subject by using Genomix (Talent Srl, Trieste, Italy). We selected 12 single-nucleotide polymorphisms (SNPs) identified as susceptibility loci for obesity by genome-wide association studies in Caucasian populations[11–13] and constructed Invader probes (Third Wave Technologies, Madison, WI, USA) for the following SNPs: rs2815752 in the neuronal growth regulator 1 gene (*NEGR1*); rs10913469 in the SEC16 homolog B gene (*SEC16B*); rs6548238 in the transmembrane protein 18 gene (*TMEM18*); rs7647305 in the ets variant 5 gene (*ETV5*); rs10938397 in the glucosamine-6-phosphate deaminase 2 gene (*GNPDA2*); rs6265 and rs925946 in the brain-derived neurotrophic factor gene (*BDNF*); rs10838738 in the mitochondrial carrier homolog 2 gene (*MTCH2*); rs7498665 in the SH2B adaptor protein 1 gene (*SH2B1*); rs1424233 in the v-maf musculo-aponeurotic fibrosarcoma oncogene homolog gene (*MAF*); and rs29941 and rs11084753 in the potassium channel tetramerisation domain-containing 15 gene (*KCTD15*). The SNPs were genotyped using Invader assays as previously described.[17] The success rate of these assays was >99.0%.

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 dominant model, homozygosity and heterozygosity with the risk allele were coded as 1 and the other was coded as 0. Multiple linear regression analyses were carried out 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 Hardy–Weinberg equilibrium was assessed using the χ^2-test.[18] Statistical analysis was carried out using the software R (http://www.r-project.org/). *P*-values were corrected by Bonferroni's adjustment 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 the Hardy–Weinberg equilibrium. The BMI, VFA and SFA values for each SNP genotype are represented in Table 3. Multiple linear regression analyses of the anthropometric parameters with respect to the 12 SNPs analyzed are shown in Table 4. No SNPs were not significantly associated with BMI in this population, although a previous study reported that the *SEC16B* rs10913469 and *TMEM18* rs6548238 SNPs were significantly associated with obesity (BMI>30 kg m^{-2}) in the Japanese population.[15]

The *SH2B1* rs7498665 SNP was significantly associated with VFA (P=0.00047) even when the conservative Bonferroni's correction was applied ($P<0.0042$). Previous reports indicate that the rs7498665 SNP is associated with waist circumference[19] or visceral fat mass[20] in the dominant model. The VFA values of the rs7498665 genotype (Table 3) suggest that the dominant model would be the best-fitted model. Therefore, we performed multiple regression analyses by using the

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 shown as mean±s.d.

Table 2 Genotypic characteristics of the subjects

SNP ID	*Nearby gene*	*Allele 1/2*	*Risk allele*	*Genotype*	*HWE P-value*
rs2815752	*NEGR1*	A/G	A	1113/163/3	0.24
rs10913469	*SEC16B*	T/C	C	690/510/78	0.20
rs6548238	*TMEM18*	T/C	C	6/201/1071	0.29
rs7647305	*ETV5*	T/C	C	201/576/500	0.10
rs10938397	*GNPDA2*	A/G	G	615/537/126	0.58
rs6265	*BDNF*	A/G (Met/Val)	G	207/609/462	0.79
rs925946	*BDNF*	T/G	T	3/100/1175	0.57
rs10838738	*MTCH2*	G/A	G	107/555/616	0.25
rs7498665	*SH2B1*	G/A (Ala/Thr)	G	29/305/945	0.46
rs1424233	*MAF*	A/G	A	726/469/82	0.59
rs29941	*KCTD15*	T/C	C	774/444/60	0.72
rs11084753	*KCTD15*	G/A	G	105/535/638	0.63

Abbreviations: HWE, Hardy–Weinberg equilibrium; SNP, single-nucleotide polymorphism.

Table 3 Mean BMI, VFA and SFA for 12 obesity-risk variants

		Mean ± s.d.								
		BMI ($kg\,m^{-2}$)			VFA (cm^2)			SFA (cm^2)		
SNP ID	Nearby gene	11	12	22	11	12	22	11	12	22
rs2815752	*NEGR1*	29.1 ± 5.9	28.3 ± 5.3	28.5 ± 3.1	124.8 ± 66.7	118.5 ± 62.6	95.2 ± 38.8	226.1 ± 103.1	228.3 ± 108.5	251.0 ± 79.8
rs10913469	*SEC16B*	28.8 ± 5.9	29.2 ± 5.6	29.7 ± 6.5	123.0 ± 66.4	124.9 ± 65.2	124.8 ± 70.4	221.7 ± 103.7	231.6 ± 102.6	234.0 ± 110.5
rs6548238	*TMEM18*	25.9 ± 7.5	29.0 ± 7.2	29.0 ± 5.5	85.9 ± 70.6	123.4 ± 67.1	124.3 ± 65.9	211.0 ± 135.0	222.8 ± 111.3	227.3 ± 102.2
rs7647305	*ETV5*	29.0 ± 5.3	29.1 ± 5.4	29.0 ± 6.3	124.5 ± 66.8	124.5 ± 66.3	123.2 ± 65.8	234.1 ± 99.5	225.6 ± 100.0	224.6 ± 109.6
rs10938397	*GNPDA2*	28.8 ± 5.9	29.1 ± 5.8	29.2 ± 5.3	122.7 ± 68.0	124.5 ± 64.0	127.4 ± 66.3	224.5 ± 103.4	227.9 ± 103.3	229.3 ± 107.5
rs6265	*BDNF*	28.6 ± 5.9	28.7 ± 5.3	29.6 ± 6.3	122.4 ± 68.2	122.9 ± 64.7	126.1 ± 67.1	220.3 ± 92.9	223.5 ± 102.2	233.2 ± 109.9
rs925946	*BDNF*	36.0 ± 10.7	29.5 ± 6.1	28.9 ± 5.7	142.6 ± 11.3	123.3 ± 63.3	124.0 ± 66.4	416.8 ± 155.7	236.3 ± 118.6	225.2 ± 101.9
rs10838738	*MTCH2*	28.7 ± 4.9	29.3 ± 6.4	28.7 ± 5.3	124.5 ± 58.3	125.1 ± 68.5	122.6 ± 65.2	214.1 ± 93.1	233.6 ± 109.6	222.2 ± 99.8
rs7498665	*SH2B1*	29.7 ± 4.8	29.5 ± 6.2	28.8 ± 5.7	134.4 ± 65.3	134.5 ± 70.5	120.2 ± 64.3	231.1 ± 95.9	235.1 ± 98.4	223.6 ± 105.5
rs1424233	*MAF*	29.0 ± 6.0	29.1 ± 5.7	28.4 ± 3.8	123.1 ± 64.7	124.0 ± 65.7	130.6 ± 80.4	222.0 ± 102.6	234.0 ± 109.1	219.7 ± 74.5
rs29941	*KCTD15*	28.8 ± 5.6	29.1 ± 6.0	30.4 ± 6.0	123.9 ± 65.2	122.4 ± 67.3	136.5 ± 68.5	224.8 ± 103.7	228.2 ± 103.5	236.6 ± 106.6
rs11084753	*KCTD15*	29.5 ± 5.7	29.1 ± 5.8	28.9 ± 5.8	128.3 ± 71.9	122.5 ± 64.9	124.5 ± 66.2	233.0 ± 98.6	227.4 ± 102.0	224.7 ± 106.1

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 in each SNP is indicated in Table 2.

Table 4 Relationship between obesity 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
rs2815752	*NEGR1*	0.611	0.448	0.17	7.423	4.847	0.13	−6.293	7.978	0.43
rs10913469	*SEC16B*	0.325	0.255	0.20	2.827	2.753	0.30	4.516	4.532	0.32
rs6548238	*TMEM18*	0.267	0.403	0.51	6.773	4.352	0.12	2.557	7.178	0.72
rs7647305	*ETV5*	0.025	0.221	0.91	1.984	2.386	0.41	2.565	3.929	0.51
rs10938397	*GNPDA2*	0.199	0.236	0.40	0.804	2.547	0.75	4.065	4.190	0.33
rs6265	*BDNF*	0.508	0.223	0.023	1.390	2.410	0.56	6.954	3.968	0.080
rs925946	*BDNF*	0.816	0.545	0.14	0.390	5.897	0.95	18.972	9.685	0.050
rs10838738	*MTCH2*	0.162	0.243	0.51	0.292	2.628	0.91	2.726	4.326	0.53
rs7498665	*SH2B1*	0.536	0.310	0.085	11.717	3.343	0.00047	8.341	5.555	0.13
rs1424233	*MAF*	0.050	0.252	0.84	−2.945	2.722	0.28	−5.311	4.479	0.24
rs29941	*KCTD15*	0.481	0.265	0.070	2.588	2.871	0.37	3.589	4.727	0.45
rs11084753	*KCTD15*	0.332	0.243	0.17	1.562	2.626	0.55	3.242	4.322	0.45

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. BMI, VFA and SFA were adjusted for age and gender.

dominant model and found a significant association between this SNP and VFA (P=0.00022). This association remained significant even after adjusting for age, gender and BMI in the dominant model (P=0.00096). The other SNPs did not show any significant association with VFA. No SNPs, including the *SH2B1* rs7498665, were associated with SFA.

BMI, VFA and SFA are known to be affected by gender; therefore, we compared the anthropometric parameters (BMI, VFA and SFA) among the different genotypes in the men and women (Supplementary Tables 1–3). Association between *SH2B1* rs7498665 SNP and VFA was not significant both in men (P=0.0099) and women (P=0.022). This negative association is most likely due to the decrease in the number of each genotype. The VFA values of the rs7498665 genotype (Supplementary Table 2) suggest that the dominant model would be the best-fitted model both in men and women. By using the dominant model, revealed no significant association between the rs7498665 genotype and VFA in men (P=0.0061) and women (P=0.015).

To confirm the association of the *SH2B1* rs7498665 SNP with VFA, two SNPs (rs4788102 and rs8049439) in linkage disequilibrium of rs7498665 reported by previous study[11] were genotyped (Supplementary Table 4). Both rs4788102 (P=0.00058) and rs8049439 (P=0.0021) SNPs were significantly associated with VFA.

DISCUSSION

In this study, we showed that the *SH2B1* rs7498665 SNP was significantly associated with VFA. Haupt *et al.*[20] used whole-body magnetic resonance imaging to show that this SNP (dominant model) was associated with visceral fat mass. They also reported that the *SH2B1* rs7498665 SNP was not associated with BMI or with non-visceral fat mass. Jamshidi *et al.*[19] reported that the *SH2B1* rs7498665 SNP (dominant model) was associated with waist circumference. Several studies have reported a negative association between the *SH2B1* rs7498665 SNP and abdominal adipose mass (measured using dual energy X-ray absorptiometry)[21] or waist circumference.[22,23]

CT- or magnetic resonance imaging-based analyses are more accurate than waist circumference- and dual energy X-ray absorptiometry-based abdominal fat-mass analysis for evaluating the association between this SNP and visceral fat mass. These data from this study and from the study performed by Haupt *et al.* strongly suggest that the *SH2B1* rs7498665 SNP is associated with visceral fat accumulation.

SH2B1 has four splicing isoforms; that is, α, β, γ and δ, of which SH2-Bβ was originally identified through its association with Janus kinase 2 (JAK2) protein, a cytoplasmic tyrosine kinase that mediates cytokine functions.[24] *SH2B1*-knockout mice have been reported to show severely impaired insulin signaling in the skeletal muscles, liver and adipose tissue, and progressively develop hyperinsulinemia, hyperglycemia and glucose intolerance.[25] *SH2B1*-knockout mice also developed hyperlipidemia, leptin resistance, hyperphagia and obesity.[26] Although data for mesenteric fat have not been reported, both subcutaneous inguinal fat and intra-abdominal (epididymal) fat were found to be increased in *SH2B1*-knockout mice.[26,27] Neuron-specific restoration of *SH2B1* in knockout mice corrected the metabolic disorders, improved leptin regulation of orexigenic neuropeptide expression in the hypothalamus, and protected against high-fat diet-induced leptin resistance and obesity.[27] Ventromedial hypothalamic lesions are reported to induce visceral fat accumulation that does not result in obesity, and to induce hyperglycemia, hyperinsulinemia and hypertriglyceridemia.[28] *SH2B1* was specifically expressed in the brain, including the hypothalamus, in mice with neuron-specific *SH2B1* restoration.[27] Therefore, *SH2B1* expression in hypothalamus (possibly the ventromedial hypothalamic) may have an important role in visceral fat accumulation. As the *SH2B1* rs7498665 SNP is a non-synonymous SNP (G/A, Ala484Thr) and exits in the proline-rich region, the function of the SH2B1 protein might be deteriorated in subjects with the risk G-allele, leading to visceral fat accumulation. The rs4788102 SNP exists in the 5′-flanking region of the *SH2B1* gene, thus, the expression of *SH2B1* may be changed in the subjects with the risk A-allele. It is necessary to investigate whether these SNPs are functional.

In summary, we showed that the *SH2B1* rs7498665 SNP is significantly associated with VFA. This SNP is not associated with BMI or SFA, suggesting that there is a VFA-specific genetic factor. Our results also suggest that the *SH2B1* gene has a role in visceral fat accumulation. However, these results need to be confirmed in other populations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This work was supported by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan (21591186) and by the Mitsui Life Science Social Welfare Foundation.

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 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).
7 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).
8 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).
9 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).
10 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).
11 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).
12 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).
13 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).
14 Hotta, K., Nakata, Y., Matsuo, T., Kamohara, S., Kotani, K., Komatsu, R. *et al.* Variations in the FTO gene are associated with severe obesity in the Japanese. *J. Hum. Genet.* **53,** 546–553 (2008).
15 Hotta, K., Nakamura, M., Nakamura, T., Matsuo, T., Nakata, Y., Kamohara, S. *et al.* Association between obesity and polymorphisms in SEC16B, TMEM18, GNPDA2, BDNF, FAIM2 and MC4R in a Japanese population. *J. Hum. Genet.* **54,** 727–731 (2009).
16 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).
17 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).
18 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).
19 Jamshidi, Y., Snieder, H., Ge, D., Spector, T. D. & O'Dell, S. D. The SH2B gene is associated with serum leptin and body fat in normal female twins. *Obesity* **15,** 5–9 (2007).
20 Haupt, A., Thamer, C., Heni, M., Machicao, F., Machann, J., Schick, F. *et al.* Novel obesity risk loci do not determine distribution of body fat depots: a whole-body MRI/MRS study. *Obesity* **18,** 1212–1217 (2010).
21 Renström, F., Payne, F., Nordström, A., Brito, E. C., Rolandsson, O., Hallmans, G. *et al.* Replication and extension of genome-wide association study results for obesity in 4923 adults from northern Sweden. *Hum. Mol. Genet.* **18,** 1489–1496 (2009).
22 Bauer, F., Elbers, C. C., Adan, R. A., Loos, R. J., Onland-Moret, N. C., Grobbee, D. E. *et al.* Obesity genes identified in genome-wide association studies are associated with adiposity measures and potentially with nutrient-specific food preference. *Am. J. Clin. Nutr.* **90,** 951–959 (2009).
23 Ng, M. C., Tam, C. H., So, W. Y., Ho, J. S., Chan, A. W., Lee, H. M. *et al.* Implication of genetic variants near NEGR1, SEC16B, TMEM18, ETV5/DGKG, GNPDA2, LIN7C/BDNF, MTCH2, BCDIN3D/FAIM2, SH2B1, FTO, MC4R, and KCTD15 with obesity and type 2 diabetes in 7705 Chinese. *J. Clin. Endocrinol. Metab.* **95,** 2418–2425 (2010).
24 Rui, L., Mathews, L. S., Hotta, K., Gustafson, T. A. & Carter-Su, C. Identification of SH2-Bbeta as a substrate of the tyrosine kinase JAK2 involved in growth hormone signaling. *Mol. Cell. Biol.* **17,** 6633–6644 (1997).
25 Duan, C., Yang, H., White, M. F. & Rui, L. Disruption of the SH2-B gene causes age-dependent insulin resistance and glucose intolerance. *Mol. Cell. Biol.* **24,** 7435–7443 (2004).
26 Ren, D., Li, M., Duan, C. & Rui, L. Identification of SH2-B as a key regulator of leptin sensitivity, energy balance, and body weight in mice. *Cell Metab.* **2,** 95–104 (2005).
27 Ren, D., Zhou, Y., Morris, D., Li, M., Li, Z. & Rui, L. Neuronal SH2B1 is essential for controlling energy and glucose homeostasis. *J. Clin. Invest.* **117,** 397–406 (2007).
28 Yoshida, S., Yamashita, S., Tokunaga, K., Yamane, M., Shinohara, E., Keno, Y. *et al.* Visceral fat accumulation and vascular complications associated with VMH lesioning of spontaneously non-insulin-dependent diabetic GK rat. *Int. J. Obes. Relat. Metab. Disord.* **20,** 909–916 (1996).

Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)

Endocrine Journal 2011, **58** (3), 193-198

ORIGINAL

Hypopituitarism in a patient with transsphenoidal cephalocele: longitudinal changes in endocrinological abnormalities

Keiji Tanimoto, Saori Onda, Hideaki Sawaki, Tetsuya Hiraiwa, Hiroyuki Sano, Mineki Ohnishi, Jungo Terasaki and Toshiaki Hanafusa

Department of Internal Medicine (I), Osaka Medical College, Takatsuki 569-8686, Japan

Abstract. We report a 21-year-old man with severe fatigue due to hypopituitarism. At the age of 6 years, he was diagnosed with short stature due to a GH deficiency accompanied by a sphenoid cystic lesion. Laboratory findings and provocative tests for pituitary hormone function revealed ACTH, LH, FSH, TSH, and GH deficiency. Computed tomography and magnetic resonance imaging revealed transsphenoidal cephalocele due to a defect in the floor of the sella turcica. At 6 years, he only had severe GH deficiency and poor response of LH to LHRH. Hypothalamic-pituitary dysfunction and pituitary herniation have progressed subsequently; we observed a longitudinal progression of hypothalamic-pituitary dysfunction caused by transsphenoidal cephalocele. This dysfunction requires the selection of a treatment that will not aggravate the condition further.

Key words: Hypothalamic-pituitary dysfunction, Panhypopituitarysm, Pituitary herniation, Follow-up, MRI

CONGENITAL basal encephaloceles are rare, with an estimated incidence of 1 in every 35,000 live births [1]. Transsphenoidal cephalocele is the least frequent anomaly, representing only 5% of basal encephaloceles (estimated incidence: 1 in 700,000 live births). In transsphenoidal cephalocele, transsphenoidal lesions collapse into the epipharynx or nasopharynx [2]. The diagnosis of transsphenoidal cephalocele is usually made in infancy or early childhood [3].

Because the floor of the sella turcica is lacking in this anomaly, hypothalamic-pituitary function may deteriorate. The natural course of hypothalamic-pituitary dysfunction due to transsphenoidal cephalocele remains unclear. There are only a few reports describing endocrinological follow-up due of transsphenoidal cephalocele for a period of 10 years or more. We report the longitudinal progression of hypothalamic-pituitary dysfunction due to transsphenoidal cephalocele in a patient. Surgical treatment for transsphenoidal cephalocele was not performed in the follow-up period; the natural course of hypothalamic-pituitary dysfunction was able to be observed.

Received Dec. 24, 2010; Accepted Jan. 19, 2011 as K10E-405
Released online in J-STAGE as advance publication Feb. 17, 2011

Correspondence to: Keiji Tanimoto, Department of Internal Medicine (I), Osaka Medical College, 2-7 Daigaku-machi, Takatsuki city, Osaka 569-8686, Japan
E-mail: in1120@poh.osaka-med.ac.jp

Case Report

A 21-year-old man was referred to our hospital with severe fatigue. At 6 years of age, he had consulted the department of pediatrics in our hospital because of his short height (97.4 cm, −3.56 SD). He was diagnosed with short stature due to GH deficiency accompanied by a sphenoid cystic lesion. Until 10 years old, he did not visit his doctor. GH replacement therapy was administered from the age of 10 to 13 years. However, the patient could not continue the GH replacement therapy after the age of 13 years. After this, he visited the doctor about only once a year, and he did not receive a treatment. At 21 years of age, he was hospitalized in a local hospital due to infectious enteritis; he was also diagnosed with secondary hypothyroidism. Following this, he was admitted to our hospital for further evaluation of hypopituitarism.

His height was 144 cm and body weight was 38 kg. He had no external malformations, but had peripapillary staphyloma in left eye. No axillary or pubic hair was present. His Tanner stages were G1 and P1, and each of his testes was 3 mL in volume. The labora-

Table 1 Laboratory findings

White blood cell	5620 /μL
Neutrophil	62.9 %
Lymphocyte	28.6 %
Eosinophil	3.2 %
Red blood cell	359×10^4 /μL
Hemoglobin	10.2 g/dL
Hematocrit	30.3 %
Platelet	25.4×10^4 /μL
Albumin	4.2 g/dL
Aspartate aminotransferase	24 IU/L
Alanine aminotransferase	13 IU/L
Lactate dehydrogenase	204 IU/L
Creatinine kinase	83 IU/L
Alkaline phosphatase	254 IU/L
Blood urea nitrogen	6 mg/dL
Creatinine	0.56 mg/dL
Uric acid	4.2 mg/dL
Total-Cholesterol	200 mg/dL
Triglyceride	105 mg/dL
Sodium	141 mEq/L
Potassium	3.9 mEq/L
Chloride	108 mEq/L
Calcium	8.9 mg/dL
Fasting serum glucose	84 mg/dL

Table 2 Endocrinological laboratory findings

		Reference value
TSH	2.790 μU/mL	(0.500~5.000)
Free T3	1.62 pg/mL	(2.30~4.30)
Free T4	0.77 ng/dL	(0.90~1.70)
PRL	11.26 ng/mL	(3.58~12.78)
GH	0.08 ng/mL	(<0.17)
IGF-I	12 ng/mL	(133~368)
ACTH	13.0 pg/mL	(7.2~63.3)
Cortisol	4.7 μg/dL	(4.0~18.3)
DHEA-S	8 μg/dL	(85~690)
UFC	8.1 μg/day	(11.2~80.3)
LH	<0.1 mIU/mL	(0.79~5.72)
FSH	0.3 mIU/mL	(2.00~8.30)
Testosterone	<0.05 ng/mL	(2.07~7.61)
Plasma Osm	279 mOsm/kg	(276~292)
Urine Osm	350 mOsm/kg	(50~1200)
ADH	1.0 pg/mL	(0.3~3.5)

Free T3: free triiodothyronine, Free T4: free thyroxine, IGF-I: insulin-like growth factor-I, ADH: antidiuretic hormone, DHEA-S: dehydroepiandrosterone sulfate, UFC: urinary free cortisol

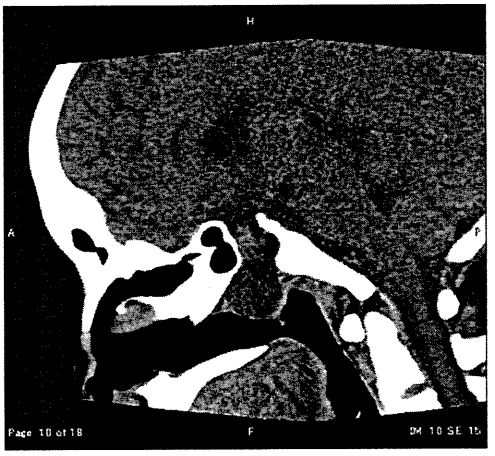

Fig. 1 Sagittal head CT demonstrated a bone defect in the floor of the sella turcica by a transsphenoidal soft tissue mass extending into the epipharynx.

tory examination is displayed in Table 1. Red blood cell, hemoglobin, and hematocrit decreased slightly. Endocrinological laboratory findings are shown in Table 2. Although the serum levels of both free triiodothyronine and free thyroxine were low, TSH was normal. IGF-I (insulin-like growth factor-I) level was low for his age and sex; IGF-I SD score was −5.7 SD. Levels of plasma ACTH, serum cortisol and dehydroepiandrosterone sulfate (DHEA-S), and urinary free cortisol were all low. LH and testosterone levels were lower than the measurable limit. Provocative tests for pituitary hormone function are shown in Table 3. The response of cortisol to 250 μg of synthetic ACTH was impaired; the peak value of cortisol was 12.9 μg/dL. Both LH and FSH levels were consistently low in response to LHRH injection. After TRH administration, TSH rose to high values, but total T3 levels did not rise enough to 1.3 times or more. These findings suggested that bioactivity of TSH is decreased in the patient. The patient had almost no response of GH after GHRP-2 (growth hormone releasing peptide-2) injection. According to hand X-rays, his bone age was equivalent to a 12.6-year-old Japanese boy. Computed tomography (CT) of the head showed a defect in the floor of the sella turcica (Fig. 1.). Magnetic resonance imaging (MRI) revealed a cystic mass extending into the epipharynx through the bone defect (Fig. 2a and 2b).

On the basis of the above mentioned findings, he was diagnosed with ACTH, LH, FSH, TSH, and GH

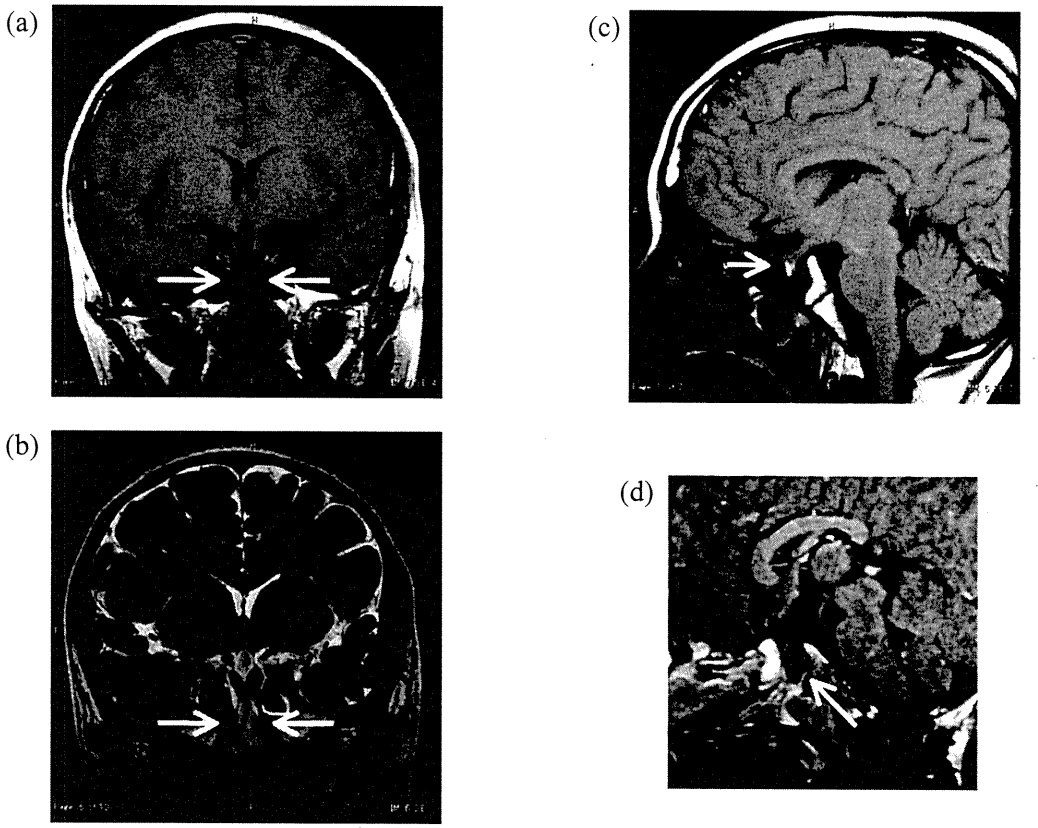

Fig. 2 T1-weighted (a) and T2-weighted (b) coronal and T1-weighted sagittal (c) MRI revealed a cystic mass with a surrounding structure extending through the bone defect into the epipharynx. The longitudinal diameter of the cystic mass was 3.5 centimeters. T1-weighted sagittal MRI at the age of 6 years (d) demonstrated a sphenoid cystic lesion 1.5 centimeters in diameter.

deficiency due to transsphenoidal cephalocele. Nasal obstruction or nasal discharge, meningitis, and cerebrospinal fluid rhinorrhea were not present; therefore, he did not undergo surgery. He received replacement therapy of adrenocortical hormone (hydrocortisone 20 mg), thyroid hormone (levothyroxine sodium hydrate 75 μg), after which his complaints were resolved. His weight was increased to 44kg, though his height did not change. Ten months after, he accepted the replacement therapy of sex hormone (testosterone enanthate 125mg, once a month) and GH (somatropin 1.3mg). However, he did not come to our hospital three months later.

Discussion

Transsphenoidal cephalocele often leads to hypothalamic-pituitary dysfunction. The incidence of hypothalamic-pituitary insufficiency was estimated at about 50–60% in previous reports [1, 4, 5]. Hypothyroidism, GH deficiency, hypogonadotrophic hypogonadism, and diabetes insipidus were frequent disorders. Central hypoadrenalism was rare in previous reports [6]. In our case, hypothyroidism, GH deficiency, hypogonadotrophic hypogonadism, and hypoadrenalism were found, but diabetes insipidus was not detected from laboratory findings and MRI (Table 2 and Fig. 2c).

We obtained the clinical examination data from the hospital where this patient had consulted a doctor in his childhood. Results of his endocrinological examination at the age of 6 years are shown in Table 4. Severe GH deficiency and poor response of LH to LHRH were observed. No evidence of ACTH, FSH, TSH, or ADH deficiency was present at that point. He was diagnosed with short stature due to GH deficiency. His subsequent clinical course is shown in Fig. 3. Free T4 level decreased gradually; nevertheless, TSH did not rise sufficiently. Testosterone levels were lower than the measurable limit at all time points. By the time he was admitted to our hospital at the age of 21, he

Table 3 Provocative tests for pituitary hormone function

	Before	15 min	30 min	45 min	60 min	90 min	120 min
ACTH (250 μg) administration test							
Cortisol (μg/dL)	2.8		11.6		12.9		
CRH (100 μg), LHRH (100 μg), and TRH (200 μg) administration test							
ACTH (pg/mL)	40.2		124		88.1	86.8	
Cortisol (μg/dL)	10.3		17.5		17.8	21.3	
LH (mIU/mL)	0.2		0.4		0.4	0.4	
FSH (mIU/mL)	0.7		1.2		1.4	1.6	
TSH (μU/mL)	8.65		27.7		25.7	21.2	
PRL (ng/mL)	20.7		28.3		22.0	17.7	
Total T3 (ng/mL)	0.75						0.97
GHRP-2 (growth hormone releasing peptide-2) (100 μg) administration test							
GH (ng/mL)	<0.03	0.12	0.08	0.04			

Table 4 Endocrinological findings when the patient was 6 years old

IGF-I	38 ng/mL	Total T4	12.9 μg/dL	Plasma Osm	279 mOsm/kg
Overnight GH	3.85 ng/mL (Mean)	Testosterone	<0.05 ng/mL	Urine Osm	799 mOsm/kg
				ADH	2.0 pg/mL

	Before	15 min	30 min	60 min	90 min	120min	150min
Insulin tolerance test (1.8U, 0.1U/kg)							
Glucose (mg/dL)	82	22	50	80	92		
GH (ng/mL)	0.41		1.4	1.2	0.3		
Cortisol (μg/dL)	11.4			26.0			
Propranolol-Glucagon administration test							
Glucose (mg/dL)	84		122	92	81	66	70
GH (ng/mL)	0.66		0.74	0.64	1.2	2.8	3.4
CRH (27 μg), LHRH (36 μg), and TRH (180 μg) administration test							
Cortisol (μg/dL)	12.6			21.5			
LH (mIU/mL)	<0.5		1.8	1.3	1.4		
FSH (mIU/mL)	0.8		4.0	4.2	5.9		
TSH (μU/mL)	2.7		13.0	8.7			
PRL (ng/mL)	4.3		13.0	7.6			

had not only GH deficiency, but also ACTH, LH, FSH, and TSH deficiency. MRI when he was 6 years old revealed a sphenoid cystic lesion 1.5 cm in diameter (Fig. 2d). The size of his transsphenoidal cephalocele had increased to 3.5 cm by the time of his admission to our hospital (Fig. 2a, 2b, and 2c). To summarize, both hypothalamic-pituitary dysfunction and pituitary herniation had progressed significantly in 15 years.

There are only a few case reports showing endocrinological follow-up for a time period of ten years or more in patients with transsphenoidal cephaloceles and pituitary herniation [2, 4, 7]. The natural course of hypothalamic-pituitary dysfunction is still unclear. Although hypothalamic-pituitary function was not necessarily examined at the first visit or during the follow-up time in all previous case reports, it has been reported that most patients (7 out of 9 patients: 77.8%) with transsphenoidal cephaloceles exhibited a progression of hormonal disturbances [8]. On the other hand, neurosurgical intervention could not effectively prevent pituitary dysfunction [4, 9]. In hypothalamic-pituitary dysfunction, surgical treatment might be required to prevent a progression of hormonal insufficiency. Since a valid surgical procedure for transsphe-

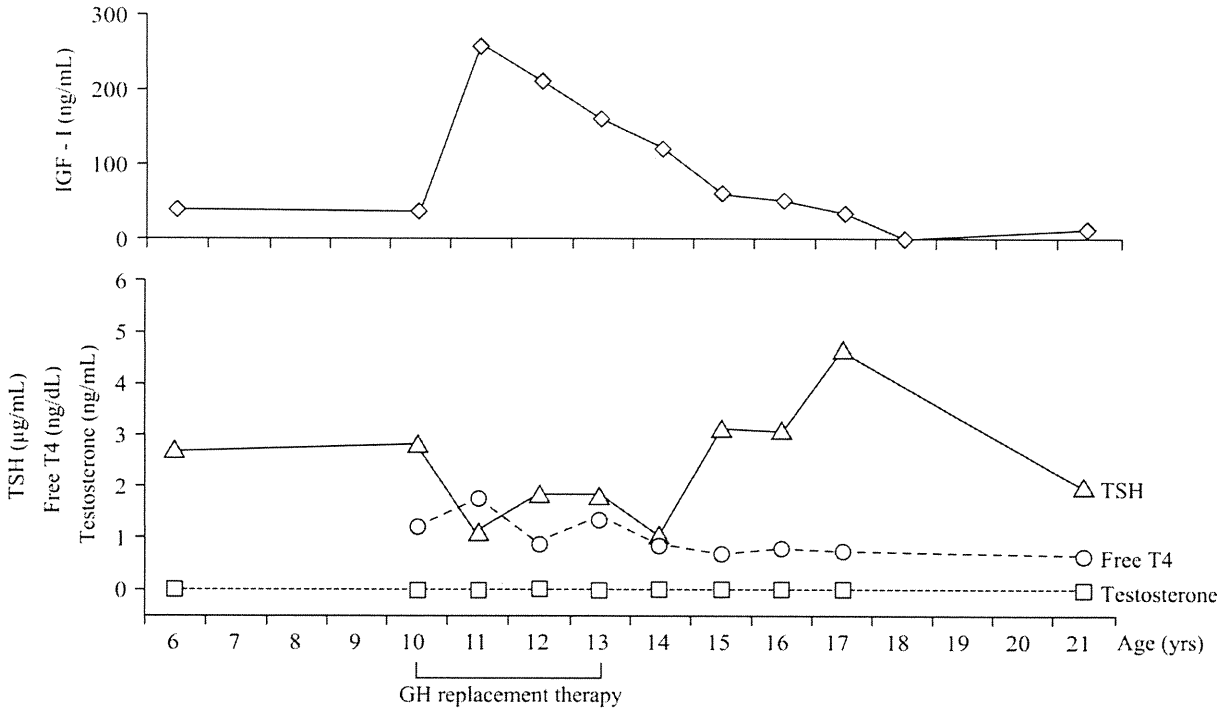

Fig. 3 Clinical course of endocrinological abnormality from 6 years to 21 years is shown. IGF-I (◇) rose temporarily in response to the GH replacement therapy. Free T4 (○) decreased gradually; nevertheless, TSH (△) did not rise appropriately. Testosterone (□) levels were lower than the measurable limit at all time points.

noidal encephalocele was recently reported, the number of treatable cases will probably increase in the near future [10-12].

The surgical treatment for transsphenoidal encephalocele has not always been beneficial [3, 13]. There is a risk of damaging the functioning tissue within the wall of encephalocele [10]. In our case, MRI revealed that a cystic mass contained a surrounding structure, and the pituitary hormones were not completely lack. In addition, surgical treatment could not effectively prevent pituitary dysfunction [4, 9]. If there was an evidence of respiratory obstruction, meningitis, cerebrospinal fluid rhinorrhea, and progressive visual defects, we will conduct a surgical repair to transsphenoidal encephalocele.

The mechanism of this hormonal abnormality is still unknown. In a child with diabetes insipidus complicated by cephalocele, the degeneration of the hypothalamus and agenesis of the supraoptic nuclei may be detected, but the description of pituitary gland anomaly had not been reported [2]. One report described a male patient with GH, TSH, and LH deficiency, and pituitary fibrosis, but a normal hypothalamus [4]. Therefore, the abnormalities of the hypothalamus and pituitary gland are variable. In the present case, MRI revealed that the periphery of the cephalocele was enhanced by gadolinium, and the posterior pituitary bright spot was observed in the sella turcica (Fig. 2). These findings suggest that both the anterior pituitary gland and the neurohypophysis existed. Accordingly, damage to the pituitary stalk might have caused hypothalamic-pituitary dysfunction in this case.

In conclusion, we have reported a patient who had ACTH, LH, FSH, TSH, and GH deficiency due to a transsphenoidal cephalocele. Both hypothalamic-pituitary dysfunction and pituitary herniation have gradually progressed in the past 15 years. In the future, surgical treatment might be a promising choice to prevent further progression of hormonal insufficiency in similar patients.

Acknowledgments

We would like to thank Dr. Kazutaka Konishi, Abuyama Pediatrics Clinic, and Dr. Okasora Keisuke, Department of Pediatrics, Hirakata Municipal Hospital, for providing the childhood clinical data of this patient.

References

1. Smith DE, Murphy MJ, Hitchon PW, Babin RW, Abu-Yousef MM (1983) Transsphenoidal encephaloceles. *Surg Neurol* 20: 471-480.
2. Pollock JA, Newton TH, Hoyt WF (1968) Transsphenoidal and transethmoidal encephaloceles. A review of clinical and roentgen features in 8 cases. *Radiology* 90: 442-453.
3. Yokota A, Matsukado Y, Fuwa I, Moroki K, Nagahiro S (1986) Anterior basal encephalocele of the neonatal and infantile period. *Neurosurgery* 19: 468-478.
4. Ellyin FM, Potchynok A, Song C (1991) Long-term follow-up of hypothalamic-pituitary function in patients with transsphenoidal pituitary herniation and midfacial anomalies. *Clin Endocrinol (Oxf)* 35: 263-266.
5. Koral K, Geffner ME, Curran JG (2000) Transsphenoidal and sphenoethmoidal encephalocele: report of two cases and review of the literature. *Australas Radiol* 44: 220-224.
6. Lieblich JM, Rosen SE, Guyda H, Reardan J, Schaaf M (1978) The syndrome of basal encephalocele and hypothalamic-pituitary dysfunction. *Ann Intern Med* 89: 910-916.
7. Durham LH, Mackenzie IJ, Miles JB (1988) Transphenoidal meningohydroencephalocoele. *Br J Neurosurg* 2: 407-409.
8. Morioka M, Marubayashi T, Masumitsu T, Miura M, Ushio Y (1995) Basal encephaloceles with morning glory syndrome, and progressive hormonal and visual disturbances: case report and review of the literature. *Brain Dev* 17: 196-201.
9. Sailer HF, Landolt AM (1987) A new method for the correction of hypertelorism with preservation of the olfactory nerve filaments. *J Craniomaxillofac Surg* 15: 122-124.
10. Tsutsumi K, Asano T, Shigeno T, Matsui T, Ito S, Kaizu H (1999) Transcranial approach for transsphenoidal encephalocele: report of two cases. *Surg Neurol* 51: 252-257.
11. Abe T, Ludecke DK, Wada A, Matsumoto K (2000) Transsphenoidal cephaloceles in adults. A report of two cases and review of the literature. *Acta Neurochir (Wien)* 142: 397-400.
12. Faggin R, Pentimalli L, Grazzini M, Saetti R, Drigo P, d'Avella D (2009) Combined endoscopic-microsurgical approach for transsphenoidal (sphenopalatine) encephalocele with an intralesional pituitary gland. Case report. *J Neurosurg Pediatr* 4: 262-265.
13. David DJ (1993) Cephaloceles: classification, pathology, and management--a review. *J Craniofac Surg* 4: 192-202.