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体脂肪減少因子を用いた２型糖尿病の治療に関する研究

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主任研究者　　武田　純

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【１】総括研究報告

厚生労働省科学研究費補助金（創薬基盤推進研究事業）
総括研究報告書

体脂肪減少因子を用いた２型糖尿病の治療に関する研究

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研究要旨

２型糖尿病は、膵β細胞のインスリン分泌不全と肥満によるインスリン抵抗性によって発症する。膵島トランスクリプトーム研究により、申請者らは、蓄積した体脂肪を減少させると共に、血糖を低下させる分泌蛋白を発見した。本研究では、同蛋白の患者血中測定による糖脂質代謝異常の診断法の開発と治療への応用を目指す。さらに、同蛋白のコード遺伝子多型を用いた疾患発症と治療の感受性の体質診断も試みる。

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A　研究目的

糖尿病に肥満の合併は高頻度であり、治療や予防において肥満の解消は重要である。特に、心血管イベントのリスク軽減において重要である。しかし、食事による減量は長期の努力を要し、十分な改善を見るに至らない場合が多い。運動療法はインスリン抵抗性の改善に欠かせないが、心機能の低下、腎障害、増殖網膜症、関節障害、リハビリなどを有する状態では、運動は困難で適応にない。一方、短期の減量には VLDC 治療が行なわれるが、入院を要する上に反動が大きい。食欲低下薬は適応が厳しく、しかも長期使用は認められていない。従って、短期に効率的な減量ができて、しかも血糖改善を同時に見込める治療は理想的である。高齢化社会ではこのような治療は患者の生活自立阻害を解消する。

糖尿病、肥満、高脂血症、動脈硬化は密接に関連した代謝病であるが、各々は個別に研究されてきた経緯がある。申請者らの糖尿病遺伝子研究により、コード蛋白は異なる発現組織（膵島、肝、脂肪細胞など）においてこれらの病態に共通に関与することが明らかとなった。このことは、慢性高血糖による二次的な病変（合併症）に加えて、一次的な共通体質が存在することを意味する。従

って、共通接点に関する分子を見出せば、新しい診断法のみならず、重要な創薬対象となる。

申請者らは、膵島トランスクリプトーム研究において、コード蛋白の内で分泌蛋白に焦点を当てた研究を行なっている。分泌蛋白を対象とした理由は、血中測定と投与が容易であり、早期の臨床応用に直結するからである。正常と糖尿病の発現プロフィールの比較解析から、幾つかの変化遺伝子に着目した。実験動物の肝で過剰発現させたところ、生理機能が不明な 32kDa 分子が体脂肪量を減少させた。興味深いことに軽度ながらインスリン分泌を亢進させて耐糖能も改善した。本研究では、肥満と糖尿病に関する診断法への開発、体質診断と治療への臨床応用を目指す。

B 研究方法

インスリン分泌に対する効果の解析

マウスインスリン産生細胞株 MIN6 に過剰発現させ、種々のグルコース濃度に対する分泌量を測定することによってインスリン合成と分泌に対する 32kDa 分子の効果を解析した。個体レベルでは、アデノウイルスによる発現系を構築したので、ラットで過剰発現させ血中レベルを上昇させて耐糖能（糖負荷試験）とインスリン分泌に関する生理学的機能を解析した。ヒトでも血中測定系を開発したので、糖尿病が疑われる症例について７５ｇ糖負荷試験を実施し、耐糖能を３群（正常型、境界型、糖尿病型）に区分して各々のグループでインスリン分泌との関連を解析した。

体脂肪量に対する効果の解析

3T3L1 細胞が脂肪細胞へ分化する過程での 32kDa 分子の発現レベルの変化を検討した。

アデノウイルス発現系を用いて腹腔内に投与し、32kDa 分子を肝で強発現させることによって血中濃度を上昇させた。体脂肪の蓄積量の変化を、褐色(BAT)と白色脂肪組織(WAT)に区分して検討した。また、種々のアディポサイトカインの分泌に対する効果も検討した。ヒトでは、血中測定により肥満や動脈硬化マーカーとの相関を解析した。

遺伝子多型と病態との関連解析

32kDa 分子のコード遺伝子のエクソンと周辺領域を全シーケンスして頻度の高い一塩基多型（SNP）を求め、SNP ハプロタイプを構築して種々の臨床表現型との関連を解析した。

倫理面への配慮

全ての実験はヘルシンキ宣言と３省庁合同指針を遵守して行われる。本計画は医学部の遺伝子解析と臨床研究に関する倫理審査委員会の承認を既に受けている。患者および健常者からの DNA と血液試料はインフォームドコンセントを取得した後に提供を受け、連結可能匿名化の状態で保存されている。匿名化 DNA はさらにプレート番号のみで表されるので二重に匿名化され、被験者のプライバシーは完全に保護される。臨床上の個人情報を含めて、研究ソースはすべて本研究に関わらない秘守義務を負う識別管理者が管理する。保存コンピュータはインターネットに連結せず、専用で独立である。

C 研究結果

インスリン分泌に対する効果の解析

32kDa 分子はラット正常膵島では高い発現が認められたが、インスリン分泌能を欠失した膵β細胞由来株 RINm5F 細胞では mRNA 発現は認められなかった。一方、GK 糖尿病ラットから抽出した膵島では３W に比して、逆に糖尿病を発症した８W では 32kDa 分子は約３倍に発現が亢進していた。この相違は現時点では不明である。

マウスインスリン産生細胞株 MIN6 に過剰発現

させると、5.5 mM と 25 mM グルコースのいずれにおいても、軽度ではあるが有意にインスリン分泌を亢進させた($p<0.01$)。正常ラットを用いた糖負荷試験では、前、３０分、１２０分のいずれにおいても血糖の低下を認めたが、対応するインスリン分泌については試料が十分ではなく次年度の課題である。HbA1c 5.5 以上のヒト 78 人について 75g 経口糖負荷試験を実施したところ、糖尿病型では全時点で低分泌が観察された。境界型と糖尿病型の症例数が十分ではなかったので、次年度はさらに症例数を増やして検討することが重要である。

体脂肪量に対する効果の解析

3T3L1 細胞を脂肪細胞に分化させると、32kDa 分子は TGF β と同程度のレベルで誘導された。そこで、脂肪細胞における 32kDa 分子の生理的機能を理解するために、アデノウイルス発現系を用いてラットの腹腔内に投与して血中に過剰発現させた。その結果、個体の体重を変化させないで、WAT と BAT 量を有意に減少させた。肝臓の重量は上昇しており、中性脂肪で占められた。以上から、32kDa 分子は体脂肪を分解して遊離脂肪酸として血中に放出し、過剰分は肝臓に取り込まれて中性脂肪として蓄積されたと推定された。

ヒトでは、BMI と中性脂肪において正の相関が認められた。空腹時血糖とは負の相関があり、空腹時インスリンとは正の相関を認めた。アディポサイトカインでは、アディポネクチンと負の相関にあり、PAI-1 とは正の相関を示した。

遺伝子多型を用いた関連解析

32kDa 分子のコード遺伝子の１０エクソンを直接シーケンスで解析したが、糖尿病の原因となる変異は同定されなかった。次いで、全域を SNP スクリーニングして頻度の高い１２SNP を見出した。連鎖不平衡解析により、２つの LD ブロックを見出した。代表 SNP を用いた糖尿病発症との関連解析の結果、有意の関連を認めた ($p<0.05$)。さらに、症例を増やすことによる確認とサブ表現型との関連解析が次年度において重要である。

D 考察

インスリン抵抗性の改善のために、２型糖尿病の予防と治療の双方に過体重の解消は重要である。動脈硬化による心血管イベントの予防にも解消は欠かせない。しかし、食事療法と運動療法を中心とした減量は不断の長期努力を要するので、続かず十分な改善に至らない場合がほとんどである。特に高齢者では、循環器疾患を既に合併していたり、整形外科的な障害のために運動制限がある場合が多い。従って、効果的な減量と耐糖能の改善を同時に見込める治療は理想的である。特に、継続的な運動やインスリン治療が困難な高齢化社会では、このような治療法は老人の生活自立阻害を予防する。一方、内蔵肥満があると軽度の耐糖能障害であっても、心血管イベントリスクとなることが明らかにされた。従って、壮年期からの健康診断による効率歴なリスクグループの早期検出と保健指導は予防策として重要である。

本研究は、これらの医療行政と社会的要請に応えるものである。

E 結論

培養細胞と動物を用いた実験レベルではあるが、本分泌蛋白は耐糖能の改善と体脂肪蓄積を減少させる機能を有すると考えられる。ヒトでの臨床調査から、実験動物とは異なる成績も見られるが類似の成績が集積されつつある。さらに、個体レベルでの解析を進めることが臨床応用にとって重要である。

F 健康危険情報

なし

G 研究発表

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(KLF11)の検討
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H　知的財産権の出願・登録

1　特許取得

なし

2　実用新案登録

なし

3　その他

なし

【２】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

雑誌

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Association of *TCF7L2* polymorphisms with susceptibility to type 2 diabetes in 4,087 Japanese subjects

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Abstract Transcription factor 7-like 2 (*TCF7L2*) has been shown to be associated with type 2 diabetes mellitus in multiple ethnic groups. Regarding the Asian population, Horikoshi et al. (Diabetologia 50:747–751, 2007) and Hayashi et al. (Diabetologia 50:980–984, 2007) reported that single nucleotide polymorphisms (SNPs) in *TCF7L2* were associated with type 2 diabetes in the Japanese population, while contradictory results were reported for Han Chinese populations. The aim of this study was to investigate the associations of the *TCF7L2* gene with type 2 diabetes using a relatively large sample size: 2,214 Japanese individuals with type 2 diabetes and 1,873 normal controls. The minor alleles of rs7903146, rs11196205, and rs12255372 showed significant associations with type 2

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Table 1 Clinical characteristics of each sample set. Data are means ± SD. *BMI* Body mass index

	Kobe		Gunma		Consortium	
	Diabetes	Control	Diabetes	Control	Diabetes	Control
n	465	323	576	576	1,173	974
Male participants (%)	59.6	45.8	56.1	40.4	56.6	43.1
Age at study (years)	60.5 ± 10.7	75.6 ± 8.1	60.2 ± 11.5	67.3 ± 6.5	62.5 ± 8.8	69.2 ± 7.0
BMI	24.3 ± 3.9	21.4 ± 3.5	23.9 ± 4.2	23.0 ± 2.9	23.1 ± 2.9	22.6 ± 3.0
HbA_{1c} (%)	8.1 ± 2.0	5.0 ± 0.4	7.8 ± 3.5	5.0 ± 0.4	7.5 ± 1.5	4.9 ± 0.4

diabetes (OR = 1.48, $P = 2.7 \times 10^{-4}$; OR = 1.39, $P = 4.6 \times 10^{-4}$; OR = 1.70, $P = 9.8 \times 10^{-5}$, respectively) in the combined sample sets. However, neither rs11196218 nor rs290487 showed a significant association. These results indicate that *TCF7L2* is an important susceptibility gene for type 2 diabetes in the Japanese population.

Keywords Type 2 diabetes · Polymorphism · β-cell function · Transcription factor 7-like 2 (*TCF7L2*) · Association study

Introduction

The transcription factor 7-like 2 gene (*TCF7L2*) is one of the most convincing susceptibility genes for type 2 diabetes. Following the initial report (Grant et al. 2006), there have been a number of association studies in various ethnic groups (Florez et al. 2006; Zhang et al. 2006; Saxena et al. 2006). Regarding the Asian population, Horikoshi et al. (2007) reported that a single nucleotide polymorphism (SNP), rs7903146, in *TCF7L2* is associated with type 2 diabetes in the Japanese population but that other SNPs (rs7895340, rs11196205, rs12255372) are not. The minor allele frequencies of these SNPs in Japanese were also found to be much lower than those of Caucasians. Hayashi et al. (2007) replicated the association of *TCF7L2* with type 2 diabetes in Japanese. Contradictory results were reported for Han Chinese populations (Ng et al. 2007; Chang et al. 2007), but these two reports found that other common SNPs (rs11196218 and rs290487, respectively) were associated with type 2 diabetes. This apparent difference between Asian populations could be due to the relatively small sample sizes involved. Recently, variants in the *TCF7L2* gene also were reported to be associated with β-cell function (Schäfer et al. 2007; Lyssenko et al. 2007) and response to sulfonylureas in Caucasians (Pearson et al. 2007). To clarify the association of the *TCF7L2* gene with type 2 diabetes and β-cell function in an Asian population, we have performed association studies using a relatively large Japanese sample set: 2,214 Japanese individuals with type 2 diabetes and 1,873 normal controls.

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Subjects and methods

Subjects

Three sample sets were involved. The Kobe set and the Gunma set samples were recruited from hospitals in Hyogo and Gunma prefecture, respectively. The Consortium set samples were recruited from seven districts in Japan by the Study Group of the Millennium Genome Project for Diabetes Mellitus. The Kobe, Gunma, and Consortium sets were independent of one another. The inclusion criteria for normal, control subjects of the Consortium set were as follows: (1) >60 years of age; (2) HbA_{1c} values <5.8%; and (3) no family history of type 2 diabetes in first- or second-degree relatives. In the Kobe and Gunma control samples, the inclusion criteria were (1) no past history of diabetes and (2) HbA_{1c} values < 5.8%. The control subjects were hospital patients for annual medical checkup or unrelated disorders. Type 2 diabetes was diagnosed in accordance with WHO criteria. Other forms of diabetes were excluded based on the clinical data. The clinical and laboratory characteristics of the study subjects are shown in Table 1. Written, informed consent was obtained from all participants. The study was approved by the ethics committee of each participating institute.

Genotyping

Five SNPs (rs7903146, rs11196205, rs12255372, rs11196218, rs290487) were genotyped using TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City,

CA) or SSP-FCS (sequence specific primer-fluorescence correlation spectroscopy) Assays (Bannai et al. 2004). Of the original five SNPs (rs7903146, rs11196205, rs12255372, rs7901695, rs7895340) in the first report (Grant et al. 2006), we selected three SNPs (rs7903146, rs11196205, rs12255372) for the following reasons: the original five SNPs are located in one linkage disequilibrium (LD) block surrounding exon 4 in the Japanese population (Supplementary Figure 1), which is similar to the case in Caucasians (Grant et al. 2006); rs7901695 and rs7895340 are in almost complete LD with rs7903146 ($r^2 = 1$) and rs11196205 ($r^2 = 0.90$), respectively, in the Japanese population (Horikoshi et al. 2007); there is no common (minor allele frequency > 10%) SNP in this LD block (HapMap JPT data). We also genotyped rs11196218 and rs290487, which were associated with type 2 diabetes in Han Chinese, to replicate this association in Japanese. To evaluate our genotyping, 180 samples in the Consortium set were genotyped by both TaqMan SNP Genotyping Assays and SSP-FCS Assays. The concordance rate between these two assays was 100%: genotypes determined by TaqMan or SSP-FCS methods were identical to those determined by direct sequencing for 48 samples.

The genotyping success rates in the three sample sets were all >93%. All five SNPs were in Hardy–Weinberg equilibrium (HWE; $P > 0.05$ in the Exact test) in both case and control groups of all sample sets.

Clinical assessment

The clinical profile of each subject was directly determined at the time of entry. HOMA-IR and HOMA-β were calculated as follows: HOMA-IR = (fasting insulin [pmol/l]) × glucose [mmol/l])/22.5 × 6 and HOMA-β = (fasting insulin [pmol/l]) × 2)/(glucose [mmol/l] − 3.5) × 6. Diabetic subjects treated with insulin were excluded from analysis of HOMA-IR and HOMA-β. Assessments were performed with the combined three sample sets. Data are expressed as means ± SD.

Statistical analysis

The differences for SNPs or estimated haplotypes between type 2 diabetic and non-diabetic subjects were compared using Chi-square test under an allelic model. We also performed multiple logistic regression analysis adjusted for age, sex, and BMI under a dominant model. Statistical analysis was performed with the Stat-View program (version 5.0-J; SAS Institute, Cary, NC). The relation of the variants in *TCF7L2* with BMI and Homeostasis model assessment (HOMA-IR and HOMA-β) by t test under the dominant model for each SNP was then assessed. The HOMA-IR and HOMA-β data were log-transformed for normality. LD and haplotype analyses were performed with SNPAlyze version 5.1 pro software (Dynacom, Mobara, Japan). We considered statistical significance at P values of < 0.01 and < 0.017 in the association study for SNPs and for clinical parameters, respectively, after Bonferroni correction. The prevalence of type 2 diabetes in the Japanese population was assumed to be 0.07. Population attributable risk (PAR) was calculated as PAR = p(RR-1)/[p(RR-1) + 1], where p and RR are the risk allele frequency in the general population and the relative risk, respectively, estimated by the prevalence. When the frequency of risk allele, OR, and type I error probability are assumed to be 0.03 (Horikoshi et al. 2007), 1.46 (Cauchi et al. 2007), and 0.05, respectively, based upon the previous study, the power of our combined samples (2,214 cases and 1,873 controls) to detect association between SNP rs7903146 and type 2 diabetes is 0.92. In the case of OR assumed to be 1.69 (Horikoshi et al. 2007), the power of our study is 0.99.

Results

We performed association analyses using three independent sample sets. Regarding three SNPs (rs7903146, rs11196205, and rs12255372), which originally showed association with type 2 diabetes, the minor alleles showed a trend toward association with type 2 diabetes in the Kobe set. These SNPs also showed a marginally significant association in the Gunma set and in the Consortium set when multiple testing was considered. In the combined three sample sets (Combined set), the minor alleles of rs7903146, rs11196205, and rs12255372 showed a significant association with susceptibility to the disease (OR = 1.48, $P = 2.7 \times 10^{-4}$; OR = 1.39, $P = 4.6 \times 10^{-4}$; OR = 1.70, $P = 9.8 \times 10^{-5}$, respectively). These associations remained significant after adjustment for age, sex, and BMI (Table 2). As in a previous report (Horikoshi et al. 2007), the MAF and PAR in our study were much lower (MAF: 0.022–0.072, PAR: ~0.02 in the Combined set) than those in Caucasians. Neither rs11196218 nor rs290487 showed a significant association in any sample set (Table 2).

LD among the five SNPs in 974 control subjects in the Consortium set was then analyzed. The D' and r^2 values are shown in Table 3. As reported previously for Japanese, three SNPs (rs7903146, rs11196205, and rs12255372) were found to be in modest to strong LD ($D' = 0.56$–1.0). Haplotypes then were constructed with these SNPs in the Combined set and assessed for association with type 2 diabetes. A haplotype comprising the risk allele of each

Table 2 Association analyses for five single nucleotide polymorphisms (SNPs) in the *TCF7L2* gene. *P* values and OR were calculated with allele data by the Chi-square test. Adjusted *P* values were calculated by multiple logistic regression (dominant model) with adjustment for age, sex and BMI. *MAF* minor allele frequency, *OR* odds ratio, *CI* confidence interval

dbSNP ID	Position on Chr10		Kobe							Gunma						
			n		MAF		OR	*P*	Adjusted *P*	*n*		MAF		OR	*P*	Adjusted *P*
			Case	Control	Case	Control	(95% CI)			Case	Control	Case	Control	(95% CI)		
rs7903146	114748339	CC	426	305	0.043	0.028	1.56	0.12	0.046	475	512	0.060	0.038	1.63	0.015	0.012
		CT	38	18			(0.89–2.76)			63	42			(1.09–2.42)		
		TT	1	0						1	0					
rs11196205	114797037	GG	408	292	0.063	0.047	1.39	0.16	0.093	455	485	0.084	0.055	1.58	0.007	0.023
		GC	55	30			(0.83–2.18)			77	58			(1.13–2.21)		
		CC	2	0						7	1					
rs12255372	114798892	GG	436	312	0.032	0.017	1.92	0.062	0.018	509	538	0.047	0.024	1.99	0.004	0.005
		GT	28	11			(0.96–3.87)			48	27			(1.24–3.20)		
		TT	1	0						2	0					
rs11196218	114830484	GG	271	194	0.23	0.23	1.01	0.92	0.23	317	334	0.22	0.22	1.04	0.72	0.84
		GA	170	106			(0.80–1.29)			184	185			(0.85–1.27)		
		AA	23	21						25	25					
rs290487	114899721	TT	181	124	0.37	0.38	0.94	0.57	0.90	209	236	0.37	0.34	1.18	0.072	0.13
		TC	226	141			(0.76–1.16)			228	235			(0.99–1.41)		
		CC	57	49						78	61					

Consortium							Combined						
n		MAF		OR	*P*	Adjusted *P*	*n*		MAF		OR	*P*	Adjusted *P*
Case	Control	Case	Control	(95% CI)			Case	Control	Case	Control	(95% CI)		
1,020	879	0.058	0.041	1.43	0.014	0.06	1,921	1,696	0.055	0.038	1.48	2.7×10^{-4}	0.0011
127	77			(1.07–1.90)			228	137			(1.20–1.84)		
3	1						5	1					
1,011	863	0.071	0.054	1.32	0.031	0.12	1,874	1,640	0.072	0.053	1.39	4.6×10^{-4}	0.0053
153	99			(1.02–1.70)			285	187			(1.16–1.67)		
6	3						15	4					
1,068	906	0.035	0.023	1.52	0.026	0.12	2,013	1,756	0.037	0.022	1.70	9.8×10^{-5}	7.0×10^{-4}
76	42			(1.05–2.21)			152	80			(1.30–2.22)		
2	1						5	1					
728	584	0.20	0.22	0.87	0.076	0.11	1,331	1,115	0.21	0.22	0.94	0.26	0.56
370	321			(0.75–1.01)			730	617			(0.85–1.05)		

Table 2 continued

Consortium						Combined					
n		MAF		OR	*P*	Adjusted *P*	*n*		MAF		OR	*P*	Adjusted *P*
Case	Control	Case	Control	(95% CI)			Case	Control	Case	Control	(95% CI)		
45	54						93	100					
476	381	0.37	0.37	0.99	0.91	0.50	873	744	0.37	0.36	1.04	0.45	0.46
507	448			(0.88–1.13)			977	824			(0.95–1.14)		
169	129						306	239					

SNP, T-C-T, was significantly associated with type 2 diabetes ($P = 5.3 \times 10^{-5}$) (Table 4).

The relation of rs7903146, rs11196205, and rs12255372 to BMI, HOMA-IR, and HOMA-β in the combined cases and controls were then compared. There was no association with BMI in cases or controls. The risk allele of rs7903146 was associated with lower HOMA-β (CC ($n = 789$) versus CT/TT ($n = 83$); 52.0 ± 87.6 versus 35.7 ± 35.9, $P = 0.009$) and lower HOMA-IR (CC vs. CT/TT; 3.2 ± 4.5 vs. 2.2 ± 1.6, $P = 0.01$) in the combined diabetic subjects. However, these associations disappeared after adjustment for age, sex, and BMI. No association was found for HOMA-β or HOMA-IR in the combined control subjects.

Discussion

We have found that three SNPs (rs7903146, rs11196205, rs12255372) of *TCF7L2* are associated with susceptibility to type 2 diabetes in the Japanese population. Our results are consistent with previous reports for Japanese populations (Horikoshi et al. 2007; Hayashi et al. 2007), but not with other reports for Han Chinese populations (Ng et al. 2007; Chang et al. 2007). The apparent difference in the association of these SNPs in Asians could be due to the low frequencies of the SNPs and the relatively small sample sizes used in the previous studies. Since we did not detect any association of rs11196218 or rs290487 in the present study, the associations of the two SNPs in the previous reports for Chinese might be specific to that population. In this study, rs7903146, rs11196205, and rs12255372 were in modest to strong LD. Based on HapMap data (JPT), the LD block surrounding exon 4 of *TCF7L2* in Asians does not exceed the gene (Supplementary Figure 1), which is consistent with findings in Caucasians (Grant et al. 2006). Previous reports (Ng et al. 2007; Chang et al. 2007) also found that the three SNPs were in a single LD block while the other two (rs11196218 and rs290487) were not. According to meta-analysis by Cauchi et al. (2007), *TCF7L2* is the most reproducible susceptibility gene for type 2 diabetes in various ethnic groups. *TCF7L2* also was one of the most significantly associated genes in recent genome-wide association studies (Sladek et al. 2007; WTCCC 2007). While the risk alleles of this gene are not common in East Asians, including Japanese, and the population attributable risk is much lower, *TCF7L2* is nevertheless a risk gene for type 2 diabetes in East Asians as well as in other populations. On the other hand, in a very recent online report, polymorphisms in the *TCF7L2* gene were found not to be associated with type 2 diabetes in a relatively large study of Pima Indians (Guo et al. 2007). Further investigation is required to

Table 3 Pairwise linkage disequilibrium (LD) for five SNPs in the *TCF7L2* gene. Values of D' (left lower) and of r^2 (upper right) for pairwise LD analysis in 974 control subjects of the Consortium set

	rs7903146	rs11196205	rs12255372	rs1196218	rs290487
rs7903146		0.24	0.49	0.002	0.0036
rs11196205	0.56		0.44	0.012	0.0037
rs12255372	0.93	1.00		0.007	0.0036
rs1196218	0.45	0.87	1.00		0.0002
rs290487	0.22	0.19	0.30	0.02	

Table 4 Association analysis for haplotypes with three SNPs (rs7903146, rs11196205, rs12255372). *P* values were calculated by the chi-square test with estimated haplotype data from the Combined set

Haplotype	Case	Control	*P*
C-G-G	0.91	0.93	1.5×10^{-4}
C-C-G	0.032	0.031	0.72
T-C-T	0.032	0.018	5.3×10^{-5}
T-G-G	0.020	0.017	0.30

elucidate the differences in the contribution of the *TCF7L2* gene to type 2 diabetes among various populations.

TCF7L2 regulates expression of the proglucagon gene (*GCG*), which encodes the precursor of glucagon, glucagon-like peptide 1 (GLP-1) (Yi et al. 2005). Several reports have found that polymorphisms of *TCF7L2* are associated with β-cell function (Florez et al. 2006; Saxena et al. 2006; Schäfer et al. 2007; Lyssenko et al. 2007). In this study, the association between the *TCF7L2* gene and HOMA-β was found to disappear after adjustment for the various factors. Although the relationship of this gene to β-cell function is not clear in this study, our results suggest that *TCF7L2* is an important susceptibility gene for type 2 diabetes in Japanese. The pathophysiological mechanism of this gene in susceptibility to type 2 diabetes remains to be elucidated.

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The ratio of leptin to adiponectin can be used as an index of insulin resistance

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Abstract

The level of leptin increases with obesity, whereas that of adiponectin decreases with obesity. It is reported that the ratio of leptin to adiponectin (L/A) is associated with insulin resistance. It is difficult to evaluate insulin resistance in diabetic patients who have a dysfunction of insulin secretion. The aim of this study was to examine whether the L/A ratio is a useful marker for insulin resistance in diabetic patients. We examined L/A in the serum of a total of 139 Japanese patients with type 2 diabetes mellitus (66 women and 73 men) and 7 healthy individuals recruited in our hospital. Changes in the levels of leptin and adiponectin were observed using the oral glucose tolerance test and a hyper- and euglycemic clamp test. Twenty-one patients with type 2 diabetes mellitus were observed for more than 6 months after treatment with pioglitazone, and 31 patients with type 2 diabetes mellitus were observed for more than 6 months after the treatment with metformin. The mean value of L/A in 139 Japanese patients with type 2 diabetes mellitus was 1.22 ± 1.41 (1.68 ± 1.76 in women, 0.81 ± 0.80 in men; $P = .0002$). In the clamp tests, L/A correlated with glucose infusion rate (GIR) ($r^2 = 0.26$, $P = .0034$). The correlation of L/A and GIR indicated a stronger correlation than either leptin ($r^2 = 0.144$, $P = .03$) or adiponectin alone ($r^2 = 0.023$, $P = .41$), or the homeostasis model assessment of insulin resistance ($r^2 = 0.103$, $P = .08$). The average hemoglobin A_{1c} (HbA_{1c}) improved from 10.2% ± 1.2% to 9.2% ± 1.6% ($P = .0037$) in 6 months after treatment with pioglitazone. Our results indicate pioglitazone to be effective for HbA_{1c} improvement in subjects with high L/A and low L/A. The average HbA_{1c} improved from 9.2% ± 0.9% to 8.0% ± 1.2% ($P = .0002$) in 6 months after treatment with metformin. Our results indicate metformin to be effective for HbA_{1c} improvement in subjects with a low L/A. In conclusion, we demonstrate that L/A is different between male and female subjects. The correlation of L/A and GIR by the euglycemic hyperinsulinemic clamp test suggests that L/A is a useful indicator for the choice of drug to treat diabetes mellitus.

1. Introduction

Obesity is defined by an accumulation of adipocytes throughout the body and is also associated with a variety of metabolic diseases. Insulin resistance or metabolic syndrome is now thought to be triggered by deposition of fat in the major target organs for insulin such as liver or muscle [1]. To improve the adverse metabolic state, it is necessary to create a negative balance in energy intake and energy consumption (ie, by exercising or by enhancing the oxidation of fatty acid in the tissues using medication), thereby leading to weight loss. Leptin and adiponectin are important hormones derived from fat cells and secreted into the serum. Both hormones improve insulin resistance [2,3], although the blood concentrations are contradictory depending on adipocyte deposition. Specifically, the level of leptin increases with obesity, whereas that of adiponectin decreases [4]. Moreover, adiponectin acts against arterial sclerosis as a "good hormone" [5]. It was recently reported that the ratio of leptin to adiponectin (L/A) could act as a useful marker for metabolic disease [6,7]. Indeed, L/A was reported to display a better correlation to insulin resistance than the level of leptin or adiponectin alone [8,9]. The ratio of leptin to adiponectin is an excellent indicator of obesity and could be a useful marker for the progression of arterial sclerosis

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