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SHORT COMMUNICATION

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Single nucleotide polymorphisms of the resistin (*RSTN*) gene

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Abstract Type 2 diabetes mellitus is a complex phenotype that is frequently associated with central obesity and insulin resistance. Recently, a protein named resistin, encoded by *RSTN* (OMIM #605565), was identified in adipose tissue. Serum resistin was elevated in obese and diabetic mice, and administration of resistin to normal mice was found to interfere with glucose tolerance and insulin action. Because of these functions, resistin is a candidate gene for diabetes and obesity. Through the use of DNA sequencing, we thus developed amplification primers for rapid screening of the *RSTN* gene that encodes resistin. No putative mutations were found, but two noncoding single-nucleotide polymorphisms (SNPs) were identified, and these were found to vary in frequency across various ethnic groups. The identification of amplification primers and SNPs provides tools to investigate resistin for association with other phenotypes.

Key words Obesity · Adipocytes · Insulin resistance · Diabetes · Genomic DNA · Sequencing · Complex traits

Introduction

Type 2 diabetes mellitus (DM) is typically characterized by central obesity and resistance of peripheral tissues to the action of insulin (Reaven 1995). Recently, a novel signaling protein, resistin, was identified by screening for molecules that were expressed during adipocyte differentiation, and also in response to an insulin-sensitizing drug (Steppan et al. 2001). Serum resistin concentrations were markedly elevated in both genetic and diet-induced murine models of obesity (Steppan et al. 2001). Immunoneutralization of resistin improved blood glucose and insulin action in murine type 2 DM (Steppan et al. 2001). In contrast, administration of resistin caused impairment of glucose tolerance and insulin action in normal mice (Steppan et al. 2001). The relevance of these murine findings to human obesity and diabetes is unknown. However, having reagents to study the human *RSTN* gene that encodes resistin on chromosome 19 (OMIM #605565) would be important to screen for mutations and to perform studies of linkage and association for diabetes or disorders of adipocyte biology such as lipodystrophy (Cao and Hegele 2000; Hegele 2000). We thus developed primers for amplification of the coding sequences of *RSTN* from genomic DNA. We report the identification of two intronic single-nucleotide polymorphisms (SNPs).

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Subjects and methods

Study subjects

We screened genomic DNA from samples taken from 21 unrelated subjects with type 2 DM and 16 unrelated normoglycemic subjects, in addition to 9 subjects with familial partial lipodystrophy but no mutation in *LMNA* encoding nuclear lamin A/C (Cao and Hegele 2000). To obtain estimates of population frequencies of the *RSTN* SNPs, we screened samples from a DNA archive of clinically normal subjects from various ethnic groups (117 Caucasians and 30 each of African, Chinese, East Indian, Amerindian, and Inuit). The study was approved by the Ethics Review Panel, University of Western Ontario.

Screening the *RSTN* gene for DNA variants

To amplify coding regions and intron–exon boundaries from genomic DNA, we developed a primer set by using GenBank sequences (Table 1). Primer sequences were derived by using GenBank accession number 12741512. There was insufficient genomic sequence data to permit the design of primers to amplify exon 1 and the promoter region of

Table 1. Amplification primers for *RSTN*

Exon(s)	Primer sequence	Product size
Exon 2	Forward 5′-GTC TCT TGG TTC CCT CTT TCA G-3′ Reverse 5′-CTG GGT TGG AGT CAG GTC TGT-3′	232 bp
Exon 3	Forward 5′-GAC CGT TTG GTC TCA CAG CTC-3′ Reverse 5′-GAC AAC AGT CTC CTG CAC TCA C-3′	180 bp
Exon 4	Forward 5′-AGA GTC CAC GCT CCT GTG TT-3′ Reverse 5′-CAT CTC CAG GTT TAT TTC CAG C-3′	249 bp

Table 2. *RSTN* gene SNP allele frequencies

Sample	Number	Intron 2 +39C>T frequency	3′-UTR +62G>A frequency
African	30	+39T: 0.18	+62A: 0.15
Caucasian	117	+39T: 0.20	+62A: 0.11
Chinese	30	+39T: 0.10	+62A: 0.13
East Indian	30	+39T: 0.30	+62A: 0.05
Amerindian	30	+39T: 0.11	+62A: 0.00
Inuit	30	+39T: 0.00	+62A: 0.00

SNP, Single-nucleotide polymorphism; UTR, untranslated region

RSTN. Primers were designed to anneal at a single temperature, which allowed for the use of a single amplification apparatus. Amplification conditions were as follows: 94°C for 5 min, followed by 30 cycles of 30 s each at 94°C, 58°C, and 72°C, and ending with a single 10-min extension step at 72°C.

Genotyping of RSTN gene SNPs

The *RSTN* +39C>T SNP in intron 2 was genotyped by amplification of a 232-bp fragment, using primers F5′-GTC TCT TGG TTC CCT CTT TCA G-3′ and R5′-CTG GGT TGG AGT CAG GTC TGT-3′, followed by electrophoresis in 2% agarose and gel purification (QIAEX II Extraction Kit, Qiagen, Mississauga ON, Canada). The purified DNA fragments were analyzed by using the Applied Biosystems Prism SNaPshot ddNTP Primer Extension Kit (PE Biosystems, Foster City, CA, USA) and the specific diagnostic primer 5′-CAA GCT CCC CAA GGG TCT-3′.

The *RSTN* 3′-untranslated region +62G>A SNP was genotyped by amplification of genomic DNA by using the primers F5′-AGA GTC CAC GCT CCT GTG TT-3′ and R5′-CAT CTC CAG GTT TAT TTC CAG C-3′. The amplification conditions were as follows: 94°C for 5 min, followed by 30 cycles of 30 s each at 94°C, 58°C, and 72°C, and ending with a single 10-min extension step at 72°C. The resulting fragment was 249 bp in length. Digestion of the +62G allele with *Bse*RI produced a single fragment of 249 bp, whereas digestion of the +62A allele produced two fragments, with lengths of 238 and 11 bp. These fragments were resolved after electrophoresis in 7% polyacrylamide gels.

Statistical analysis

SAS version 6.12 (SAS Institute, Cary, NC, USA) was used for statistical analyses. Allele frequencies were determined from electropheretogram tracings of genomic DNA sequences, except for two SNPs assayed by using restriction digestion. Chi-square analysis tested the deviation of genotype frequencies from Hardy-Weinberg predictions. The nominal level of significance for statistical analyses was $P < 0.05$.

Results

Identification of SNPs

Genomic DNA sequencing found two SNPs, designated *RSTN* +39C>T, which was 39 bp downstream of the boundary of exon 2 and intron 2, and +62G>A, which was 62 bp downstream of the last base of the codon for termination in the 3′-untranslated region of exon 4. No other *RSTN* variants were found.

SNP frequencies

The observed genotype frequencies of all SNPs did not deviate from Hardy-Weinberg expectations. Allele frequencies are shown in Table 2.

Discussion

We report (1) definition of primer sets to amplify the coding sequences of the *RSTN* gene; and (2) the use of these amplification primers for genomic DNA sequencing, which has resulted in (3) the identification of two novel SNPs. In addition, we have ruled out the presence of coding sequence mutations in the *RSTN* gene among subjects with lipodystrophy without mutations in *LMNA*. Of 534 *RSTN* alleles from various ethnic groups, we found a range of allele fre-

quencies, including the absence of both minor alleles from an Inuit sample. We were not able to examine the *RSTN* promoter region, and therefore we cannot absolutely rule out the presence of such SNPs. The reagents described in this report could be helpful to screen the *RSTN* gene when it becomes a candidate for other phenotypes, through positional cloning, analogy with other phenotypes, or expression data showing changes in response to particular experimental interventions. The SNPs could also be helpful in linkage mapping and for association studies.

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