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SHORT COMMUNICATION

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Single-nucleotide polymorphisms of the proprotein convertase subtilisin/kexin type 5 (*PCSK5*) gene

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Abstract The proprotein convertase, subtilisin/kexin type 5, or PCSK5, mediates post-translational endoproteolytic processing for several integrin α subunits. We identified two silent single-nucleotide polymorphisms (SNPs) in *PCSK5*, which were found to vary in frequency across ethnic groups. The identification of these amplification primers and SNPs provides tools to investigate *PCSK5* for association with inflammatory or vascular phenotypes.

Key words Inflammation · Cardiovascular disease · Genomic DNA · Sequencing · Complex traits

Introduction

Proprotein convertases are calcium-dependent serine proteases related to bacterial subtilisins and to yeast kexin (Seidah and Chretien 1999). These enzymes process precursor proteins to their active forms by selective cleavage of the polypeptide at sites following paired basic amino acids. In mammals, this family comprises PCSK1, PCSK2, PCSK4, PCSK5, furin, and PACE4 (Seidah and Chretien 1999). Substrates for these enzymes range from precursors for growth factors to cell surface receptors and viral surface glycoproteins. For instance, several integrin α subunits undergo post translational endoproteolytic processing at pairs of basic amino acids that is mediated by the proprotein convertase, subtilisin/kexin type 5, or PCSK5 (Lissitzky et al. 2000). *PCSK5* (OMIM #600488) encodes this proprotein convertase, which has been mapped to 9q21.3 (van de Loo et al. 1996). We used our established approach (Cao and Hegele 2000) to develop amplification primers for *PCSK5* coding sequences from genomic DNA and report the identification of two single-nucleotide polymorphisms (SNPs).

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Methods

Study subjects

We screened genomic DNA from samples taken from 16 normal unrelated subjects. To estimate population frequencies of the *PCSK5* SNPs, we screened 226 alleles from clinically normal subjects from various ethnic groups (66 Caucasian, 60 Chinese, and 50 each of African and Amerindian). The study was approved by the Ethics Review Panel, University of Western Ontario.

Screening the *PCSK5* gene for DNA variants

To amplify coding regions and intron–exon boundaries from genomic DNA, we developed a primer set using GenBank sequences (see Table 1). Primer sequences were derived using GenBank accession numbers XM_011818 and 13641334. Primers were designed to anneal at a single temperature, which allowed for use of a single amplification apparatus. Amplification conditions were 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. Amplified fragments were purified and directly sequenced (ABI 377 Prism, ABI, Mississauga, ON, Canada).

Genotyping of *PCSK5* gene SNPs

The *PCSK5* 1191G > A SNP was genotyped using amplification with primers for exon 3, as shown in Table 1. The resulting fragment was 350 bp in length. Digestion of the 1191G allele with *Dde*I produced four fragments with length 221, 75, 31, and 23 bp, whereas digestion of the 1191A allele produced five fragments, with length 119, 102, 75, 31, and 23 bp. These fragments were resolved by electrophoresis in 2% agarose gels.

Table 1. Amplification primers for *PCSK5*

Exon	Primer sequence	Product size (bp)
Exon 1	F 5′- ATT CTT ACC TTG TCT TTC CCC A -3′ R 5′- AAA GAT CAA GCT CTT TGT TTG TCA -3′	260
Exon 2	F 5′- TGA GTG ACG TTA TAA TTT GAC CTA TG -3′ R 5′- CTC TAT TTT GGG GCA TTG TCA T -3′	227
Exon 3	F 5′- TAT TTA TTC CAT GCC TCC AGG T -3′ R 5′- AGC AGC TGT TCC CTT CTA CCT A -3′	350
Exon 4	F 5′- CAT TGG ATT TGA AAT ATC GCA C -3′ R 5′- CTG GCC AAA ATA TAC TCC ATC A -3′	251
Exon 5	F 5′- TTG GTC ATG CTG TGA TTT CAT T -3′ R 5′- TTT CAT TCC ATT CCA TTC CAT T -3′	274
Exon 6	F 5′- ATT CAA GGT AAA GCT GAC CTG C -3′ R 5′- GCA CAC ACC CCA TGG TAT TAG -3′	255
Exon 7	F 5′- CTC AGC ACC TCC AAG AGA ATG -3′ R 5′- GGT CAC TCA AAT GAA AAC ACC A -3′	332
Exon 8	F 5′- TGC TTT TAT GGA ATC CAA TTG AC -3′ R 5′- ATT TAC ATT GAG AAG CTC TTT GGG -3′	200
Exon 9	F 5′- AGT CAT TAC GTT TTG GCC TCA T -3′ R 5′- CCA AAC CAG AAA GAA AAG CAA A -3′	223
Exon 10	F 5′- ATT CTT TGA CGC CAT TTT CTC A -3′ R 5′- AGG GGG CAG ATC CAC TTA CT -3′	250
Exon 11	F 5′- AGT AAA CAC ACC TTC CCC TGT G -3′ R 5′- AAG TGG AAA AAT ATT AGT CCT GAA AG -3′	256
Exon 12	F 5′- TTC CCC AAA TCT GCC TCT CT -3′ R 5′- ACA GGA ACA AAG AAA TCT AAG ATG G -3′	250

The *PCSK5* 2007C > T SNP was genotyped by amplification with primers for exon 9, as shown in Table 1. The resulting fragment was 223 bp in length. Digestion of the 2007C allele with *Alw*21I produced two fragments with length 141 and 82 bp, whereas digestion of the 2007T allele produced one fragment, with length 223 bp. These fragments were resolved after electrophoresis in 2% agarose 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 sequence, except for two SNPs that were assayed 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

SNP identification

Genomic DNA sequencing found two silent coding SNPs, designated *PCSK5* 1191G > A in exon 3, and 2007C > T in exon 9. No other variants were found.

SNP frequencies

The observed genotype frequencies of all SNPs did not deviate from Hardy–Weinberg expectations, and allele frequencies are shown in Table 2. The *PCSK5* exon 9 2007T allele was absent from Chinese and Amerindian study samples.

Table 2. *PCSK5* gene single-nucleotide polymorphism allele frequencies

Sample	Number	Exon 3 1191A frequency	Exon 9 2007T frequency
African	50	0.16	0.04
Caucasian	66	0.27	0.17
Chinese	60	0.38	0.00
Amerindian	50	0.41	0.00

Discussion

We report (1) the definition of primer sets to amplify the coding sequences of the *PCSK5* gene; and (2) the use of these amplification primers for genomic DNA sequencing, which has resulted in (3) the identification of two novel SNPs. The reagents described in this report could be helpful to screen the *PCSK5* gene when it becomes a candidate for phenotypes that involve inflammation or the cardiovascular system, 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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