J Hum Genet (2002) 47:611–613

SHORT COMMUNICATION

Jian Wang · Xiao-Yan Wen · A. Keith Stewart
Robert A. Hegele

Polymorphisms in the gene encoding phosphatidylserine-specific phospholipase A1 (*PSPLA1*)

Received: June 28, 2002 / Accepted: July 22, 2002

Abstract We report the identification of nine DNA polymorphisms in *PSPLA1* encoding phosphatidylserine-specific phospholipase A1. Because the gene product shares homology with triglyceride lipases, these polymorphisms may be useful in association studies with metabolic traits such as hypertriglyceridemia and related lipoprotein abnormalities, and will also provide tools for mapping studies of chromosome 3q13.13–13.2.

Key words Metabolism · Triglyceride · Lipase · Genomic DNA · Sequencing · Complex traits

Introduction

Phosphatidylserine-specific phospholipase A1 (PSPLA1), a serine phospholipid-specific phospholipase, was initially cloned from rat platelets (Sato et al. 1997). Although *PSPLA1* belongs to the phospholipase gene family, the predicted amino acid sequence of the gene product demonstrated greater homology with mammalian triglyceride lipases than with phospholipases (Sato et al. 1997). Human *PSPLA1* was cloned and mapped to chromosome 3q13.13–13.2 (Nagai et al. 1999), and the murine homolog was cloned and mapped to mouse chromosome 16 (Wen et al. 2001). To have tools to evaluate a possible role for *PSPLA1* in lipoprotein or metabolic disorders, we characterized intron–exon boundaries of human *PSPLA1*, developed primers to amplify coding regions of *PSPLA1* from genomic DNA, and discovered nine DNA polymorphisms.

J. Wang · R.A. Hegele (✉)
Blackburn Cardiovascular Genetics Laboratory, Robarts Research Institute, 406-100 Perth Drive, London, ON N6A 5K8, Canada
Tel. +1-519-663-3461; Fax +1-519-663-3037
e-mail: hegele@robarts.ca

X.-Y. Wen · A.K. Stewart
Department of Experimental Therapeutics, Toronto General Research Institute, University Health Network, Toronto, ON, Canada

Subjects and methods

Study subjects

All study subjects were of Caucasian descent. We screened for polymorphisms in 10 subjects with hypertriglyceridemia and 10 clinically normal subjects. The 10 screened hypertriglyceridemic subjects were a random subset of 120 unrelated dyslipidemic subjects (63 women), who had mean (±standard deviation) age, body mass index, plasma total cholesterol and high-density lipoprotein cholesterol, and plasma triglyceride level of 51.3 ± 12.4 years, 33.0 ± 5.2 kg/m^2, 7.51 ± 2.52 mmol/l, 1.04 ± 0.23 mmol/l, and 6.88 + 5.56 mmol/l, respectively. The 10 screened normolipidemic subjects came from a sample of 137 age- and sex-matched normolipidemic unrelated matched subjects of Caucasian descent for calculation of polymorphism allele frequencies. The University of Western Ontario Ethics Review Panel approved the study.

Screening candidate genes by DNA sequencing

To amplify coding regions and intron–exon boundaries, we characterized intron–exon boundaries for all exons except exon 1 using GenBank accession numbers 13644358 and AF035268. Amplification primers were then designed to include at least 50 nucleotides within each intron at each intron–exon boundary, and primer sequences are shown in Table 1. Amplification conditions for all reactions were 94°C for 5 min, followed by 30 cycles of 30 s each of denaturation at 94°C, followed by a unique annealing temperature (Table 1) and extension at 72°C, and ending with a final single 10-min extension step at 72°C. Purified amplification fragments were sequenced using an Applied Biosystems Prism 377 Automated DNA Sequencer (Mississauga, Ontario, Canada), and electropherograms were interpreted using Sequence Navigator software (version 1).

Table 1. *PSPLA1* amplification primers

Exon	Primer sequence	Product size (bp)	tanneal (°C)
1	F: GAT TTC CAG CTC AGC G R: TTC ACC TCA GAA GAG CGA	212	60
2	F: CCT TCT AAG AAC AGA TGA CCT CAC R: TCT CCT TGT TGC CCA CTA ACT TC	273	62
3	F: GAC AAT GAA TAT CCA CTT GGC A R: TTT GTC TTT CTG ACC CTC TCT C	301	62
4	F: TTT CCC TCC AAA GTA AGT GTG GTG R: TTC AGG GCA AGC TTT CTC TAT CTC	301	62
5	F: TCT ATG ATC TGC ACT GGA AGG GTG R: ACT GTG CAA CAG AAG CTC AGA GAG	297	62
6	F: GAA ATC TGT CTG AGG GCT CAT CC R: AAG GAC ACT GTC AAG TAG ACC CTC	350	62
7	F: ATG CAT GTA TTT ACA GCT TGC TGG R: GGA TAG AAT TCT TGA CCA TAG GAC	384	62
8	F: ATA CAG CAC ATA GTG CGT GTA TGG R: GCC TTT GCT CTT ATC ATT CAC TCC	331	59
9	F: CAC ACA GCC ACT AAG AGA TAG AGC R: CAT GTG TCT GTG GCT GGA TCT A	390	59
10	F: TTG GTG CAT GAC AGC CAA CCA R: TGG TCT TTG ATG TGT GAG TGC ATC	329	62
11	F: GAT TTC TTT CAC CAG ACA TCA R: ATT GAA TCC TAA GTA CGA ATG	526	62

Table 2. *PSPLA1* DNA polymorphisms

			Caucasian allele frequencies	
Exon	Nucleotide change	Amino acid	Control	Dyslipidemia
Coding sequence polymorphisms				
3	360G>A	R110H	A: 0.043	0.033
3	343T>C	F104	C: 0.15	0.10
5	604C>T	P191	T: 0.13	0.10
5	622C>G	T197	G: 0.15	0.08
8	1036G>A	P335	A: 0.10	0.17
Noncoding sequence polymorphisms				
2	−12delC		Deletion: 0.15	0.10
4	IVS4+29-+35delGCTTTAG		Deletion: 0.13	0.15
5	+77G>A		A: 0.15	0.08
6	+38G>A		A: 0.45	0.53

Polymorphism genotyping

Genotypes of all *PSPLA1* polymorphisms were determined from electropherogram tracings of direct sequencing experiments in subsamples of 40 control subjects and 40 dyslipidemic subjects, using specific amplification conditions for the culprit exon as outlined earlier and in Table 2. Chi-square analysis was used to assess deviation of genotype frequencies from Hardy-Weinberg expectations, and to compare frequencies of the single-nucleotide polymorphism (SNP) in the dyslipidemic and normal samples. This sample size afforded 74% power (alpha = 0.05, two-sided) to detect a 2.5-fold difference in allele frequency between cases and controls for allele frequency of 0.2. For the 360G>A (R110H) SNP, all 120 dyslipidemic subjects and 137 normolipidemic control subjects were genotyped using direct genomic DNA sequencing. This sample size afforded 74% power (alpha = 0.05, two-sided) to detect a 2.5-fold difference in allele frequency between cases and controls for an allele frequency of 0.04.

Results

Identification of PSPLA1 *polymorphisms. PSPLA1* genomic structure and position of polymorphisms are shown in Fig. 1. Genomic DNA sequencing experiments in normal controls and dyslipidemic subjects uncovered nine *PSPLA1* polymorphisms (Table 2). There were five coding sequence and four noncoding sequence polymorphisms (Table 2). The observed frequency of each genotype did not deviate from Hardy-Weinberg expectations (all $P > 0.50$). Caucasian allele frequencies from subsets of 40 normolipidemic subjects and 40 dyslipidemic subjects are shown. The allele

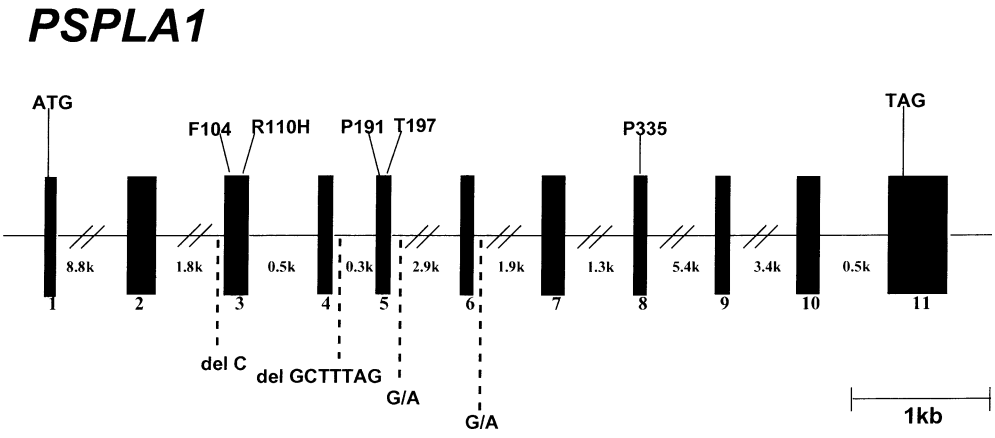

Fig. 1. Genomic structure of *PSPLA1* and position of polymorphisms. The figure shows the 11 exons of *PSPLA1*, with the intron sizes indicated *below the line* representing the gene. The start site of transcription is shown. The positions of the coding sequence polymorphism from Table 2 are shown in the *upper half* of the figure, with the affected codons and amino acids indicated. The positions of the intron polymorphisms are shown *below the gene*. The scale is shown at the *bottom right*

frequencies were not significantly different (all $P > 0.40$). In an expanded analysis of 120 subjects with dyslipidemia and 137 normal subjects, the 360A allele frequency was 0.033 in dyslipidemic subjects and 0.045 in normolipidemic subjects ($P = 0.65$).

Discussion

Hypertriglyceridemia and associated lipoprotein abnormalities are important determinants of atherosclerosis (Busch and Hegele 2000). Although mutations in the genes encoding lipoprotein lipase and hepatic lipase underlie some rare familial syndromes, the molecular defects in most patients with elevated triglycerides are unknown (Busch and Hegele 2000). *PSPLA1* is an interesting candidate gene for plasma triglyceride variation, based on its homology with other human triglyceride lipases (Nagai et al. 1997; Sato et al. 1999). Our findings suggest that variation in *PSPLA1* is not associated with hypertriglyceridemia, although the sample size we studied was admittedly relatively small. Much larger sample sizes may be required to detect more modest associations, especially for polymorphisms whose minor allele frequency is low. The identification of amplification primers and DNA polymorphisms provides tools for a more systematic assessment of the association between variation in *PSPLA1* and plasma triglycerides, or related metabolic traits. The DNA polymorphisms also provide tools for mapping studies of chromosome 3q13.13–13.2.

Acknowledgments Pearl Campbell provided outstanding technical assistance. Dr. Hegele holds a Canada Research Chair (Tier I) in Human Genetics and a Career Investigator award from the Heart and Stroke Foundation of Ontario. This work was supported by grants from Canadian Institutes for Health Research (MT13430), the Heart and Stroke Foundation of Ontario, the Canadian Genetic Diseases Network, and the Blackburn Group.

References

Busch CP, Hegele RA (2000) Variation of candidate genes in triglyceride metabolism. J Cardiovasc Risk 7:309–315

Nagai Y, Aoki J, Sato T, Amano K, Matsuda Y, Arai H, Inoue K (1999) An alternative splicing form of phosphatidylserine-specific phospholipase A1 that exhibits lysophosphatidyl-serine-specific lysophospholipase activity in humans. J Biol Chem 274:11053–11059

Sato T, Aoki J, Nagai Y, Dohmae N, Takio K, Doi T, Arai H, Inoue K (1997) Serine phospholipid-specific phospholipase A that is secreted from activated platelets: a new member of the lipase family. J Biol Chem 272:2192–2198

Wen XY, Stewart AK, Skaug J, Wei E, Tsui LC (2001) Murine phosphatidylserine-specific phospholipase A1 (Ps-pla1) maps to chromosome 16 but is distinct from the lpd (lipid defect) locus. Mamm Genome 12:129–132