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SHORT COMMUNICATION

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DNA polymorphisms of lipase related genes

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Abstract We previously determined sequenced the *PNLIP* gene encoding pancreatic lipase in cell lines of subjects with clinical deficiency of pancreatic lipase (MIM 246600) and found no putative disease-causing mutations. As part of the ongoing analysis of the genomic DNA of these subjects, we now report the development of genomic amplification primers to sequence the coding regions of *CLPS*, *CEL*, *PLRP1*, and *PLRP2*, encoding pancreatic co-lipase, carboxyl-ester lipase, and pancreatic-lipase-related proteins-1 and -2, respectively. Whereas we found no putative disease-causing missense or nonsense mutations in these samples, we discovered a total of 13 common polymorphisms (12 single nucleotide polymorphisms) in these four genes. Genotypes of these polymorphisms may be useful in future association analyses.

Keywords Lipolysis · Metabolism · Lipids · Complex traits

Introduction

Pancreatic lipase deficiency (PLD; MIM 246600) is characterized by malabsorption of long chain triglyceride fatty acids, failure to thrive in infancy and childhood, and the absence of pancreatic lipase (PL) secretion after secretin stimulation (Sheldon 1964; Rey et al. 1966). We have previously shown that the *PNLIP* gene encoding PL is not mutated in PLD (Hegele et al. 2001). Other candidate genes for PLD include those with related function, such as *CLPS* and *CEL*, which encode pancreatic co-lipase (Sims and Lowe 1992) and carboxyl-ester lipase (Lidberg et al. 1992), respectively, and with related structures, such as *PLRP1* and *PLRP2*, which encode PL-related proteins-1 and -2, respectively (Giller et al. 1992). We sequenced these four candidate genes in subjects with PLD. Whereas no putative disease-causing mutations were discovered, we found several common polymorphisms that we further characterized in normal subjects.

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Materials and methods

Study subjects

Cell lines of PL-deficient probands

Lymphoblasts from four PLD patients were purchased from Coriell Cell Repositories (Camden, N.J.). These subjects have been previously reported (Hegele et al. 2001). Briefly, subject GM13278 was a 3-year-old Caucasian female with chronic diarrhea, failure to thrive, and complete deficiency of PL activity following secretin stimulation. Subject GM13322 was a 2-year-old Hispanic male with failure to thrive, developmental abnormalities, and complete deficiency of PL activity following secretin stimulation. Subject GM13343 was a 5-year-old Caucasian male with chronic diarrhea, failure to thrive, and complete deficiency of PL activity following secretin stimulation. Subject GM13344 was a 2-year-old Caucasian male with failure to thrive, developmental abnormalities, and complete deficiency of PL activity following secretin stimulation. No family data were available for any subject.

Normal controls

Samples from a DNA archive of 50 normal Caucasian subjects were used to determine polymorphism allele frequencies. Five of these normal control DNA samples were included in the screening experiments. Samples were available from 50 Chinese and 50 African subjects for additional genotyping of polymorphisms.

Cell culture and genomic DNA isolation

Lymphoblasts were cultured in minimal essential medium (Life Technologies, Rockville, Md.) supplemented with 20% heat-inactivated fetal bovine serum at 37°C in an atmosphere of 5% CO_2 and 95% air. Genomic DNA from cultured lymphoblasts was obtained by using a commercial method, following the manufac-

Table 1 Amplification primers for genes encoding lipase-related proteins (*MIM* Mendelian Inheritance in Man, *NCBI* National Center for Biotechnology Information)

Gene	MIM number	NCBI number(s)	Region	Primer sequences	Product size
CLPS	120105	NM_001832 and NT_007592	Exon 1	F: 5' CCA CCC ACA CCA GCT GTC R: 5' CAA GAT GCC ACT CCT CCC	230 bp
			Exon 2	F: 5' GAG AAG GGA CCT GAG GGA GT R: 5' CCC TGA TCC CAC TCA CAA GT	250 bp
			Exon 3	F: 5' AGG GGA CAA GTG ATG TCC AA R: 5' CTG GAG CAG GGG AGA GAT G	273 bp
CEL	114840	NT_031835 and NM_001807	5' promoter	F: 5' AGT GTC TCG GTG AGG GTG AC R: 5' ACT TCT GGG ACA GAA AGG GC	513 bp
			3' promoter	F: 5' CCC AGG AAA AGC TCG TAC A R: 5' ATG GTG AGC ATC AGC CTC TG	459 bp
			Exon 1	F: 5' TGA GAG CTC ATG AAC AAG CAT R: 5' CCC GAA GTT CCA GAT CTG ATT	221 bp
			Exons 2 + 3	F: 5' CTG ACC CTG CCC GTG TCT R: 5' GAC TCA AGC CAG GAG TAG ACC	459 bp
			Exon 4	F: 5' CTT GGA GCA CCT CCC TGT CT R: 5' AGG GGT GCT GAG GAA AAT CT	388 bp
			Exon 5	F: 5' ACT GGT CTC TAG CAC CCC CT R: 5' CCC GTT TGA GCT TCC TCT C	227 bp
			Exon 6 + 7	F: 5' CCT TGG TGA CGG GAT TTC T R: 5' CAA CCC CCA CCT CGA GAC	445 bp
			Exon 8 + 9	F: 5' CTG CTA ACC TGC TGG CTC TC R: 5' GCT GCC CAG ATC CTC ACT T	633 bp
			Exon 10	F: 5' CAG TGA GCA CCC TGC CTA C R: 5' CTG GCT GTG CAC GTG CAT GGG C	431bp
			5' exon 11	F: 5' CCT GCA CAG CCT CTT CTC AC R: 5' CAG GGG TGG CCT CCT GGT	221 bp
			3' exon 11	F: 5' ACC AGG AGG CCA CCC CTG R: 5' CAG GAA GAG CTC AAG ATG GG	647 bp
PLRP1	604422	NM_006229 and NT_008902	Exon 1	F: 5' AAA GGG CTG GAC ACT CGA T R: 5' GTT TAA GCC CTT CCT ACG GG	276 bp
			Exon 2	F: 5' CAG AGC ACA CCT TGA GAG CA R: 5' GGA GGA AGA GGG CAC AGA G	302 bp
			Exon 3	F: 5' GAG ATG AGG AAG GAA AAA GGC R: 5' TGC ATA AAG TGA TGC AGA GAT G	270 bp
			Exon 4	F: 5' AAG TGG CCA TTT CTG AGC C R: 5' AAA CAG AGA CCA GCA GGC AC	275 bp
			Exon 5	F: 5' ATC CAT TAG GCT GGC ATT TT R: 5' TTC AGG GGA GCT CTG AAC TG	269 bp
			Exons 6 + 7	F: 5' ATA GCC CGG GCA GCT GTA R: 5' TTG CTG CAG AAA GGT ATT GG	493 bp
			Exon 8	F: 5' CCA TCC CAT TTG GAG AGA GA R: 5' AGG CAT TGC AAA TCC CAA	283 bp
			Exon 9	F: 5' GGA AAA TTG AGT GTC TGC CC R: 5' AGC AAG CCC CTC ATT TGT AA	286 bp
			Exon 10	F: 5' TCT TAT GCT TTG TTG CCT CAG A R: 5' TGT GTG GTT TGA AGG CTG TG	272 bp
			Exon 11	F: 5' CAG CCT GGC ATT GTA TGG TA R: 5' CAC GTA ACT TTC TAG GGT GGG	320 bp
			Exon 12	F: 5' ACC AAA CCA AAC CAA ACC AA R: 5' CAG ATG CAC CAG TGG ATT TTA	209 bp
PLRP2	604423	NT_008902 and NM_005396	Exon 1	F: 5' CTC ACT GTC CTT GTG ACC CG R: 5' CCC AGG CAT GTC ACT TTC TC	200 bp
			Exon 2	F: 5' TTC CAG AAG TTC ACA CAT GTC C R: 5' GGG CAG TGA CTT ATG CCC TA	288 bp
			Exon 3	F: 5' TGG CTC TAA TAG ACT CGT GGC R: 5' GCT GGT CTC ACC AGG GAG T	231 bp
			Exon 4	F: 5' GTG AAT GCC TAT CAC TGG GG R: 5' CTC TCT GAC CCC TAC CCA CA	256 bp
			Exon 5	F: 5' GAC CTG ACA CGC TAT CCC TC R: 5' CCG AGG CTT CCG AGG TG	201 bp
			Exon 6	F: 5' TCC CAG TTC TTA AGA GTC CAG C R: 5' GAG GGG ATA TCT GGA CCC AA	234 bp
			Exon 7	F: 5' ACT TTG GCA CCA GTC ACC TC R: 5' TGT CAG CAT TAT GAA GGC CA	253 bp
			Exon 8	F: 5' TGA AAG GCC TCC TTT GAG C R: 5' CAG GAA CAC AAA AAC ACC CC	227 bp

Table 1 (Continued)

Gene	MIM number	NCBI number(s)	Region	Primer sequences	Product size
			Exon 9	F: 5' AGC TGA ATT GAT TGA CTA ACA GAC A R: 5' TTA TTC GCA AGA ATT TGA AGT GA	220 bp
			Exon 10	F: 5' TTC AAT AAG ATG AGT TCA TTA AAT GGG R: 5' ATT CAG TGG TTC TGT ATA ATA GAT GCC	270 bp
			Exon 11	F: 5' TGA TAG ATC GCA ATC ATG TTA TGT R: 5' GGG CAA TTG GAT GAA TGA AT	323 bp
			Exon 12	F: 5' TGC CCA AAT GCT GTT TAC TC R: 5' GCA TTA AAG ATT TTA TTA CCG CAA	184 bp

Table 2 Polymorphisms in genes encoding lipase-related proteins (*c*. cDNA sequence nucleotide number, *IVS* intron, *PAGE* polyacrylamide gel electrophoresis, *ins* insertion, *rs1049125* identification number in National Center for Biotechnology Information SNP database)

Gene	Region	Polymorphism name	Detection method	Allele frequencies
CLPS	Exon 1	−3A → G	*Hph*I digestion	−3G in Caucasians: 0.40 −3G in Chinese: 0.07 −3G in Africans: 0.23
CEL	Promoter	−121G → T	Direct sequencing	−121T in Caucasians: 0.18 −121T in Chinese: 0.48 −121T in Africans: 0.13
		−90G → T	Direct sequencing	−90T in Caucasians: 0.23 −90T in Chinese: 0.48 −90T in Africans: 0.48
	Exon 9	c.1254C → T	*Dde*I digestion	1254T in Caucasians: 0.13 1254T in Chinese: 0.25 1254T in Africans: 0.19
		c.1316C → T(T409I)	*Bsm*AI digestion	I409 in Caucasians: 0.22 I409 in Chinese: 0.18 I409 in African: 0.17
PLRP1	IVS 8	+20C → T	*Xho*I	+20T in Africans: 0.23 +20T in Caucasians: 0 +20T in Chinese: 0.01
	IVS 9	−14C → T	Allele-specific method	−14C in Caucasians: 0.02 −14C in Chinese: 0 −14C in Africans: 0
	IVS 11	−55 insAAAAC	PAGE fragment size	insAAAAC in Caucasians: 0.37 insAAAAC in Chinese: 0.05 insAAAAC in Africans: 0
	Exon 12	c.1401T → C(L461P; rs1049125)	*Bbv*I digestion	1401C in Caucasians: 0.16 1401C in Chinese: 0.15 1401C in Africans: 0.03
PLRP2	Exon 5	c.474A → G	*Bfa*I digestion	474G in Caucasians: 0.76 474G in Chinese: 1 474G in Africans: 1
	Exon 10	c.1071A → G(W357X)	*Mbo*I digestion	1071G in Caucasians: 0.53 1071G in Chinese: 0.33 1071G in Africans: 0.55
	Exon 10	c.1081A → G(I361 V)	*Eco*RV digestion	1081G in Caucasians: 0.48 1081G in Chinese: 0.33 1081G in Africans: 0.55
	Exon 10	c.1158A → G	*Taq*I digestion	1158G in Caucasians: 0.40 1158G in Chinese: 0.33 1158G in Africans: 0.15

turer's instructions (Puregene, Gentra Systems, Minneapolis, Minn.). Genomic DNA from neonatal samples was prepared as described (Hegele et al. 2001).

Screening candidate genes for DNA variants

In order to amplify *CLPS*, *CEL*, *PLRP1*, and *PLRP2* coding regions and intron-exon boundaries from genomic DNA, primer sets were developed by using NCBI sequences. Gene names, OMIM numbers, National Center for Biotechnology Information (NCBI) numbers, primer sequences, and product sizes are shown in Table 1. Primers were each designed to anneal at 60°C, which allowed for the use of a single amplification apparatus for all reactions. Amplification conditions were: 94°C for 5 min, followed by 30 cycles comprising 30 s each at 94°C, 60°C, and 72°C, ending with a single 10-min extension step at 72°C. All samples were directly sequenced in a Prism 377 Automated DNA sequencer (PE Applied Biosystems, Mississauga, Canada).

Genotyping candidate gene polymorphisms

Rapid genotyping methods included: (1) detection of length polymorphism either by gel electrophoresis of amplified fragments for insertion/deletion polymorphisms or by digestion with a restriction endonuclease followed by gel electrophoresis for polymorphisms that altered a recognition site; (2) the use of a fluorescent allele-specific detection method (SNaPSHOT, PE Applied Biosystems, Mississauga, Canada); (3) the use of electropherograms of direct sequencing reactions. The details of the methods used for each specific polymorphism are shown in Table 2.

Statistical analysis

SAS version 6.12 (SAS Institute, Cary, N.C.) was used for all statistical analyses. Chi-square analysis was used to test deviations of genotype frequencies from those predicted by the Hardy-Weinberg equation. The nominal level of statistical significance for all analyses was $P < 0.05$.

Results

Identification of candidate gene polymorphisms

Genomic DNA from the cell lines from the four PLD subjects and from five normal Caucasian controls were screened by using the amplification primers from Table 1 and the amplification program described above. The polymorphisms detected in the screening experiments are shown in Table 2. Frequencies of the minor allele of each polymorphism in 50 Caucasians are shown in Table 2, in addition to frequencies of specific alleles in 50 Chinese and 50 African subjects. None of the observed genotype frequencies deviated from the expectations according to the Hardy-Weinberg equation.

Discussion

We report: (1) primer sets that amplify the entire sequence of *CLPS*, *CEL*, *PLRP1*, and *PLRP2*, encoding pancreatic co-lipase, carboxyl-ester lipase, and pancreatic-lipase-related proteins-1 and -2, respectively; (2) no mutations in these four genes in subjects with PLD; (3) a total of 13 common polymorphisms, 12 being single nucleotide polymorphisms (SNPs), in these four genes. Of the 12 SNPs, 11 appear not to have been previously reported, whereas only *PLRP1* L461P has been cited in the NCBI SNP database (no. rs1049125). The *PLRP1* IVS8 $+20C \rightarrow T$ SNP was first identified in subject GM13322 and appears to be specific for subjects of African origin, suggesting that subject GM13322 might have had African admixture. The *PLRP2* W357X SNP is also of particular interest since it is a common nonsense polymorphism that predicts premature truncation, by ~24%, of the gene product, whose full length form is expressed and has lipase activity (Giller et al. 1992). However, in the absence of evidence of expression, functional data, or additional clinical correlation, the relevance of such a truncated gene product is uncertain. The common SNPs in these genes may prove to be helpful in future association and/or pharmacogenetic studies.

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