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SHORT COMMUNICATION

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Cloning and identification of the human LPAAT-zeta gene, a novel member of the lysophosphatidic acid acyltransferase family

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Abstract Lysophosphatidic acid (LPA) is a naturally occurring component of phospholipid and plays a critical role in the regulation of many physiological and pathophysiological processes including cell growth, survival, and pro-angiogenesis. LPA is converted to phosphatidic acid by the action of lysophosphatidic acid acyltransferase (LPAAT). Five members of the LPAAT gene family have been detected in humans to date. Here, we report the identification of a novel LPAAT member, which is designated as *LPAAT-ζ*. *LPAAT-ζ* was predicted to encode a protein consisting of 456 amino acid residues with a signal peptide sequence and the acyltransferase domain. Northern blot analysis showed that LPAAT-ζ was ubiquitously expressed in all 16 human tissues examined, with levels in the skeletal muscle, heart, and testis being relatively high and in the lung being relatively low. The human *LPAAT-ζ* gene consisted of 13 exons and is positioned at chromosome 8p11.21.

Keywords Lysophosphatidic acid acyltransferase · Tissue expression pattern

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Introduction

Lysophosphatidic acid (LPA) is the simplest form of naturally occurring phospholipids and is also known to have a growth-factor-like activity in the regulation of numerous cellular responses through the activation of specific G-protein-coupled receptors. LPA is present in several biological fluids (serum, plasma, and aqueous humor) and is produced in variety types of cells including platelets, fibroblasts, adipocytes, and cancers.

LPA production is associated with a number of diseases, such as cancer and injuries, and may be involved in the pathogenesis of cancer, obesity, and arteriosclerosis. Cellular responses regulated by LPA include mitogenesis, cell adhesion, cytoskeletal rearrangements, ion transport, cell differentiation, smooth muscle contraction, and apoptosis (Moolenaar 1995). Recently, LPA was recognized as a diagnostic marker for ovarian cancer.

Whereas LPA is well known as a lipid growth factor, it was originally considered as a membrane component and a metabolic intermediate in lipid biosynthesis. In cells, LPA is converted to phosphatidic acid (PA), a precursor in the biosynthesis of all the species of glycerolipid, by the action of lysophosphatidic acid acyltransferase (LPAAT; EC 2.3.1.51, 1-acylglycerol-3-phosphate acyltransferase; Kent 1995). PA is also involved in phospholipid signal transduction and is rapidly increased upon cellular activation and mitogenesis (Brindley and Waggoner 1996; English et al. 1996). In the membrane of the endoplasmic reticulum, the conversion rate of LPA to PA is especially high. Consistent with this phenomenon, LPAAT is more concentrated in microsomes and in the plasma membrane. Further researches have revealed that LPAAT activity may be regulated by phosphorylation in response to interleukin-1 (Soling et al. 1989; Bursten et al. 1991).

The genes coding for LPAAT have been characterized in non-mammalian and mammalian species. Bio-

informatic approaches have facilitated the cloning of human genes as substantial amounts of information and resources have accumulated during the progress of the human genome project. Five human LPAAT genes have been identified to date, two of which have been fully characterized as having enzymatic activity (Eberhardt et al. 1997; Stamps et al. 1997; West et al. 1997; Aguado and Campbell 1998). In this report, we describe the identification of a novel human LPAAT gene. This study should facilitate research on LPAAT and its substrate.

Materials and methods

Sequence data mining

A BLAST (basic local alignment search tool) search of the GenBank database with the mouse LPAAT sequence (AF015811) revealed a series of human expressed sequence tags (ESTs: BE312159, BE545487, BE730936, etc) that had homology to the mouse query. These ESTs were then assembled resulting in the formation of a 2592-bp contig by the ContigExpress program in the Vector NTI software package.

Cloning and sequencing

Based on the EST contig sequence, two oligonucleotide primers (forward: 5'-GCTGGCCTGGCCTGGATCTTC-3'; reverse: 5'-GAGTCTGGAAAGGACACAGCAG-3') were synthesized in order to obtain a long open reading frame (ORF) in the assembled sequence, by using a human testis cDNA library (GibcoBRL) as a template in a polymerase chain reaction (PCR). The 1490-bp DNA fragment by PCR was then isolated from low-melting-temperature agarose and subjected to direct sequencing with a Q377 automated sequencer (ABI). The PCR product was also subcloned into a pGEM-T vector (Promega) and verified by nucleotide sequencing.

Northern blotting

Northern blots containing poly(A)-selected human RNA isolated from 16 tissues (heart, brain, placenta, lung, liver, muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon, and peripheral blood leukocytes) were purchased from CLONTECH (Palo Alto, Calif.). A DNA segment containing the *LPAAT-ζ* ORF was used as a probe after labeling with [^{32}P]-dATP (Random Primers Labeling Kit, Amersham). Hybridization was carried out as previously described (Tu et al. 2000). *β*-Actin cDNA was used as a control.

Results

PCR cloning of LPAAT-ζ cDNA

We first searched human EST sequences in public databases for those having homology to a mouse LPAAT sequence (AF015811) and constructed a possible contig by assembling several ESTs. A 1490-bp cDNA fragment was obtained by PCR with the designed primers and cDNA as a template (see above). The fragment contained a 1368-bp ORF, which encoded a predicted protein of 456 amino acid residues with a calculated molecular mass of 52.1 kDa (Fig. 1A). This novel gene was designated as *LPAAT-ζ* based on its amino acid sequence homology to other members of human LPAAT family (see below).

Genomic structure around the *LPAAT-ζ* gene

With the determined human *LPAAT-ζ* cDNA sequence, we searched human genomic sequences in the Human Genome Working Draft database (http://genome.ucsc.edu/) and found that the human *LPAAT-ζ* gene consisted of 13 exons and 12 introns and spanned about 122 kb at chromosome 8p11.21 (Fig. 1B). All the intron-exon boundaries followed the "GT-AG" rule (Fig. 1C). The STS markers (D8S464, D8S482, and D8S392) were localized near the *LPAAT-ζ* gene, and the *ANK1* gene (NM_000037) encoding Ankyrin 1 was situated adjacent to *LPAAT-ζ* (Fig. 1B).

Protein properties of LPAAT-ζ

LPAAT-ζ shared 11%–16% identical amino acid residues with the other five members of the human LPAAT family. Within the acyltransferase domain, the identity increased to the 17%–21% range (Fig. 2A). The predicted enzymatic domain of LPAAT-ζ was in the region of amino acids 234–403. In addition, LPAAT-ζ contained a predicted signal peptide sequence in the region of amino acids 1–37 (Nielsen et al. 1997). The calculated pI value of the LPAAT-ζ protein was 9.28. Like other human LPAAT members, the LPAAT-ζ protein was predicted to have multiple hydrophobic regions, which may serve as transmembrane regions (Fig. 2B). Phylogenetic analysis showed that LPAAT-α and LPAAT-*β* had a close relationship, with LPAAT-*γ* and LPAAT-*ϵ* exhibiting another close relationship. LPAAT-ζ was relatively distinct among the members in the phylogenic relationship (Fig. 2C).

Tissue expression pattern of human LPAAT-ζ

Northern blot analysis demonstrated that human LPAAT-ζ mRNA was ubiquitously expressed in all the 16 human tissues tested, but that the levels of its expression varied in respective tissues. Higher expression was detected in skeletal muscle, heart, and testis, while lower levels were found in the brain, lung, thymus, small intestine, colon, and peripheral blood leukocytes (Fig. 3). Three transcripts with 3 kb, 4.3 kb, and 4.6 kb were detected in the tissues examined, and an additional transcript of 2.7 kb was detected in the testis.

Discussion

The initial step of phospholipid biosynthesis involves the acylation of glycerol-3-phosphate (G3P) at the *sn*-1

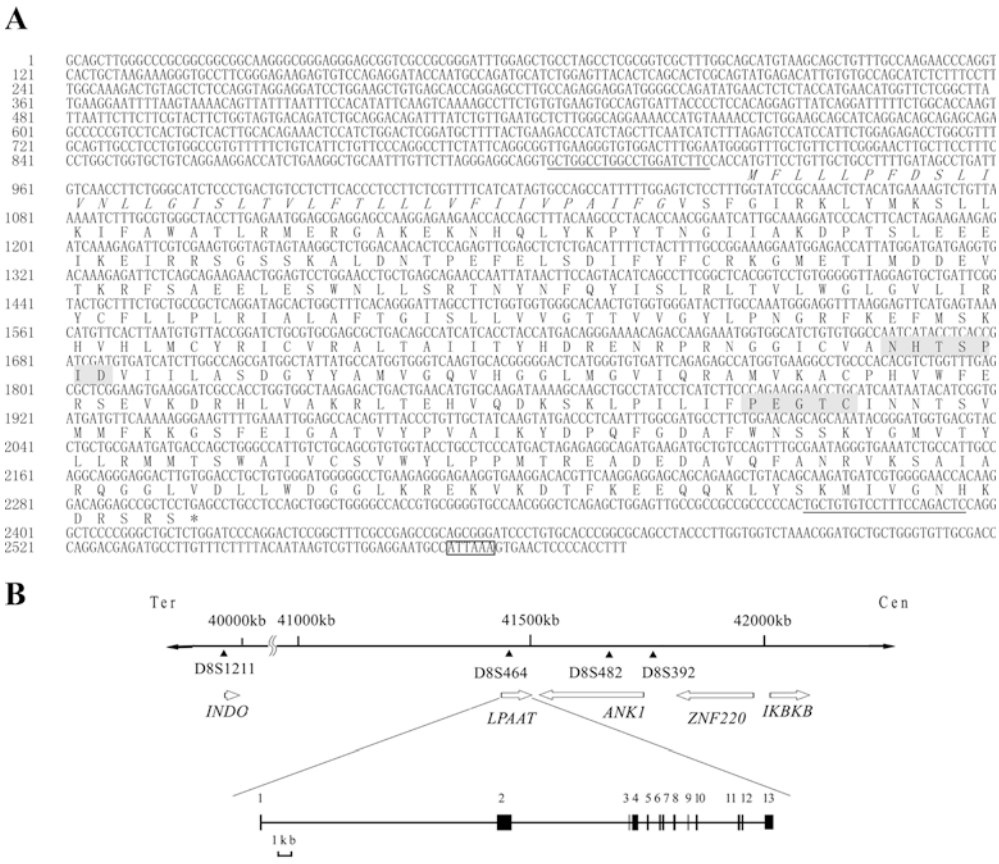

Fig. 1A–C Human LPAAT-ζ cDNA sequence and its genomic structure. **A** The nucleotide and putative amino acid sequences of human *LPAAT-ζ*. The sequences *underlined* are the primers used to clone the DNA segment (*boxed letters* poly(A) signal, *asterisk* stop codon). The conserved motifs of N**HT**SxxD and **PEG**TC are shown in gray. The predicted signal peptide is indicated in *italics*. **B** *Top* Genomic organization around *LPAAT-ζ*. This result was obtained after BLAST searches with the Human Genome Working Draft database (http://genome.ucsc.edu/). *Triangles* STS markers, *arrows* human *LPAAT-ζ* and its neighboring genes. *Bottom* Exon distribution of human *LPAAT-ζ*. **C** Junctions between introns and exons of *LPAAT-ζ*. The size and positions of exons are shown

C

Exon	Length of exon (bp)	Position in Chromosome 8	Sequence
1	79	41464725–41464803	GCAGC……GGTCGgtgag
2	1013	41484817–41485829	cccagCTTTG……TTGCGgtaag
3	70	41495941–19563792	cccagTGGGC……CAACGgtaag
4	301	41496180–41496480	tgcagGAATC……CTCAGgtgag
5	75	41497261–41497335	tacagGATAG……GGGAGgtgag
6	90	41498428–41498507	tgcagGTTTA……GACAGgtgag
7	94	41498705–41498798	cccagGGAAA……CCATGgtaag
8	116	41499370–41499485	cttagGTGGG……AAGAGgtaat
9	56	41500911–41500966	gaaagACTGA……AGAAGgtaag
10	86	41501488–41501573	tttagGAACC……TCAAGgtata
11	129	41505209–41505337	ttcagTATGA……GAGAGgtgag
12	80	41505423–41505502	tacagGCAGA……CTGTGgtaag
13	403	41507418–41507820	tccagGGATG……CCTTT

position by G3P acyltransferase to form LPA. The second step involves the acylation of LPA at the *sn*-2 position by LPAAT to form PA. LPA and PA are two phospholipids involved in signal transduction and in lipid biosynthesis in cells. As a key enzyme in glycerophospholipid biosynthesis, LPAAT has been extensively characterized in both non-mammalian and mammalian species. We have characterized the sixth member of the human LPAAT family.

Previous sequence alignment of LPAAT proteins from various species shows that the regions around the amino acid sequences NHQSxxD and PEGTR, which occur at amino acids 103–109 and 177–181 of human LPAAT-α respectively, are the most conserved sequences (Leung 2001). Site-directed mutagenesis has shown that the conserved His and Asp residues within the motif N**H**QSxx**D** and the Glu and Gly residues within the motif **PEG**TR are essential for catalytic

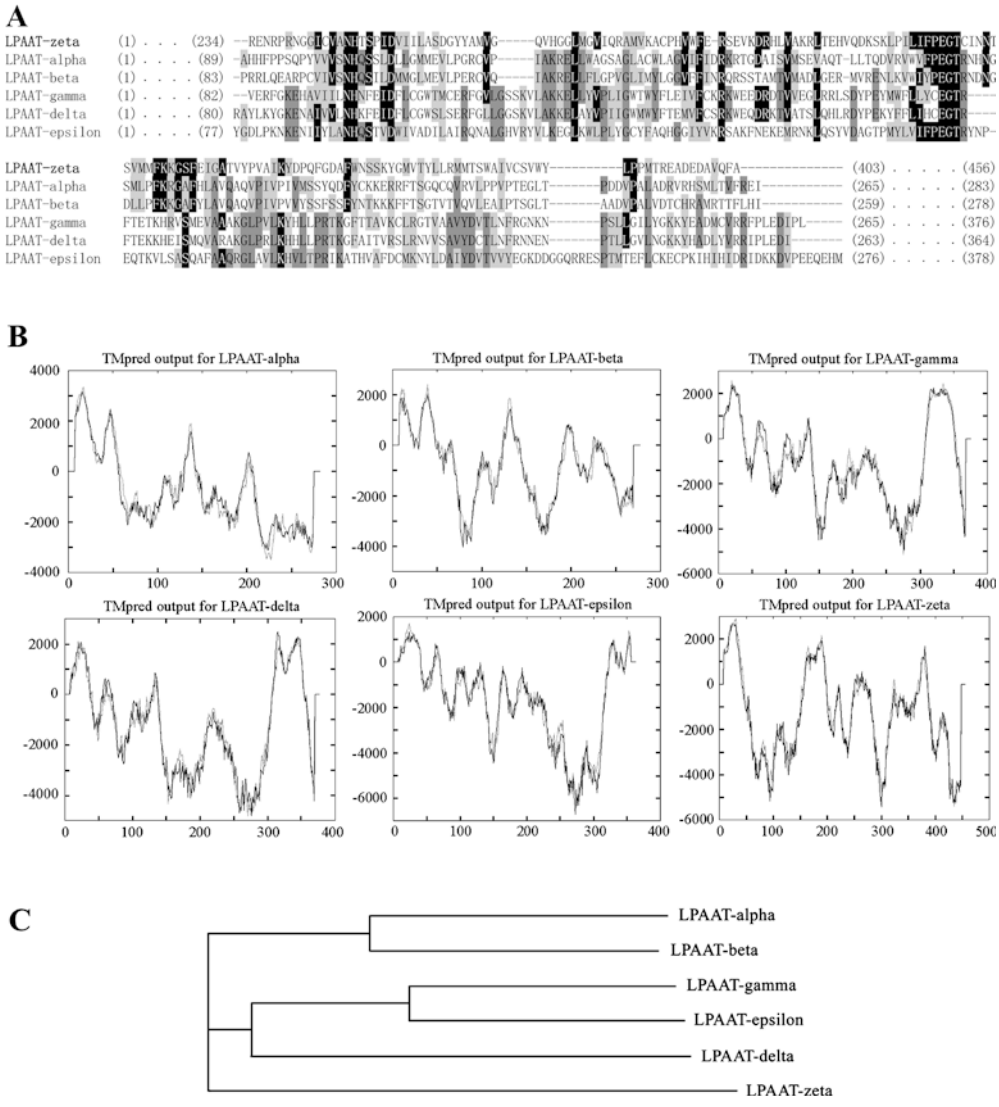

Fig. 2A–C Amino acid sequence similarity in the enzyme domain and hydrophobic profiles among six human LPAAT members. **A** The amino acid alignment of the enzyme domains of the six human LPAATs was configured by the Align X program in VectorNTI Software. GenBank accession numbers are as follows: LPAAT-alpha, U56417; LPAAT-beta, U56418; LPAAT-gamma, AF156774; LPAAT-delta, AF156776; LPAAT-epsilon, AF375789; LPAAT-zeta, AF406612. Identical amino acid residues in the family are marked in *black*, conservative residues are given in *dark gray*, and similar blocks are shown in *light gray*. *Dashes* and *spaces* introduced to optimize the alignment, *dots* omitted amino acid residues outside of the domain. **B** Transmembrane profiles of the six LPAAT members were predicted by the TMpred program. **C** Phylogenic tree of six members of the human LPAAT family

function (Lewin et al. 1999; Heath and Rock 1998). Although the identical amino acid residues do not occupy any given position among all the six members of the family, the LPAAT-ζ protein nevertheless contains sequence similarities within these two core regions. The corresponding sequences are **NHTSPID** and **PEGTC** at amino acids 247–253 and 321–325 respectively.

All six members of the human LPAAT family appear to contain a structural motif of four transmembrane regions (Fig. 2B). Although the position of the four transmembrane regions are not exactly matched among the members, and the profiles of the four transmembrane regions in LPAAT-α and β and in LPAAT-γ and ϵ are similar. This is consistent with the phylogenic relationship between the six human LPAAT members (Fig. 2C).

Among the five human LPAAT members reported previously, only LPAAT-α and β have been characterized with respect to enzymatic activity (West et al. 1997; Eberhardt et al. 1997; Stamps et al. 1997; Aguado and Campbell 1998). Although the other three members (LPAAT-γ, δ, and ϵ) have been cited in a review (Leung 2001) and deposited in public sequence databases, confirmation is lacking whether they have enzymatic activity or not. This is also the case with our LPAAT-ζ; we will report this elsewhere, after completion of characterization.

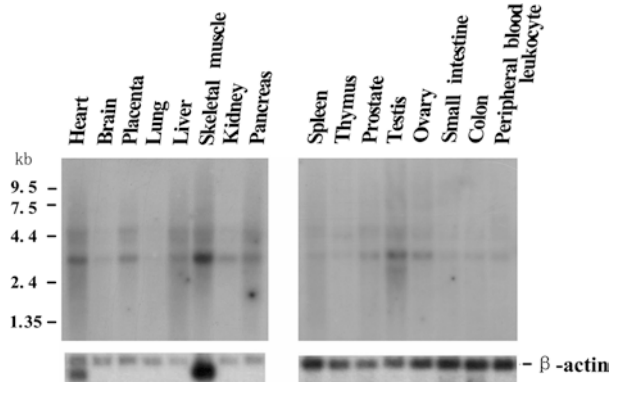

Fig. 3 Expression of LPAAT-ζ in human adult tissues. β-Actin was used as a control. *Numbers left* RNA size markers (in kb)

Northern blot analysis showed that LPAAT-ζ was expressed in all the 16 human tissues tested, with the highest expression level being found in skeletal muscle. Three transcripts of 3 kb, 4.3 kb, and 4.6 kb were detected in all tissues examined, a result that may attributable to the alternative utilization of polyadenylation signals at the 3'-untranslated region. The transcript with a smaller size was detected exclusively in the testis; this may be a tissue-specific splicing variant. According to previous reports (Eberhardt et al.1997, West 1997), LPAAT-α and β were also ubiquitously expressed in various human tissues. However, the relative expression level in the respective tissues varied among LPAAT-α, β and ζ. LPAAT-α was abundantly expressed in the heart, brain placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, lymph node, thymus, appendix, peripheral blood lymphocytes, and bone marrow, and was undectable in fetal liver. LPAAT-β was highly expressed in the liver, pancreas, heart, small intestine, lung, liver, and skeletal muscle, moderately expressed in the spleen, thymus, prostate, testis, ovary, colon, peripheral blood lymphocytes, and kidney, and slightly expressed in the placenta and the certain regions of the brain. Both LPAAT-α and β generated only one transcript. The different expression profiles among the LPAAT members suggest that these three genes are individually regulated and are likely to have independent functions.

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