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Comparative studies of vertebrate lipoprotein lipase: A key enzyme of very low density lipoprotein metabolism

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ABSTRACT

Lipoprotein lipase (LIPL or LPL; E.C.3.1.1.34) serves a dual function as a triglyceride lipase of circulating chylomicrons and very-low-density lipoproteins (VLDL) and facilitates receptor-mediated lipoprotein uptake into heart, muscle and adipose tissue. Comparative LPL amino acid sequences and protein structures and *LPL* gene locations were examined using data from several vertebrate genome projects. Mammalian *LPL* genes usually contained 9 coding exons on the positive strand. Vertebrate LPL sequences shared 58–99% identity as compared with 33–49% sequence identities with other vascular triglyceride lipases, hepatic lipase (HL) and endothelial lipase (EL). Two human LPL N-glycosylation sites were conserved among seven predicted sites for the vertebrate LPL sequences examined. Sequence alignments, key amino acid residues and conserved predicted secondary and tertiary structures were also studied. A CpG island was identified within the 5′-untranslated region of the human *LPL* gene which may contribute to the higher than average (×4.5 times) level of expression reported. Phylogenetic analyses examined the relationships and potential evolutionary origins of vertebrate lipase genes, *LPL*, *LIPG* (encoding EL) and *LIPC* (encoding HL) which suggested that these have been derived from gene duplication events of an ancestral neutral lipase gene, prior to the appearance of fish during vertebrate evolution. Comparative divergence rates for these vertebrate sequences indicated that *LPL* is evolving more slowly (2–3 times) than for *LIPC* and *LIPG* genes and proteins.

1. Introduction

Lipoprotein lipase (LPL or LIPL; E.C.3.1.1.34) is one of three members of the triglyceride lipase family that contributes to vascular lipoprotein degradation and plays major roles in hydrolyzing circulating chylomicrons and very-low-density lipoproteins (VLDL) and in facilitating receptor-mediated lipoprotein uptake into heart, muscle and adipose tissue of the body (Wion et al., 1987; Dichek et al., 1991; Benlian et al., 1996). Hepatic lipase (HL; gene *LIPC*; E.C. 3.1.1.3) also serves a dual role in triglyceride hydrolysis and in ligand-binding for receptor-mediated lipoprotein uptake into the liver (Martin et al., 1988; Datta et al., 1988; Cai et al., 1989; Holmes et al., 2011a) whereas endothelial lipase (EL; gene *LIPG*; E.C.3.1.1.3) functions in high density lipoprotein (HDL) hydrolysis in the body (Jaye et al., 1999; Hirata et al., 1999; Holmes et al., 2011b). These enzymes are members of the vascular lipase gene family which have significant sequence similarities (Hirata et al., 1999; Ma et al., 2003; Brown and Rader, 2007).

The gene encoding LPL (*LPL* or *LIPL*) is expressed in various cells and tissues of the body, including heart, muscle, adipose tissue, brain, macrophages, lung, lactating mammary gland and endothelial cells where the enzyme hydrolyzes triglycerides from chylomicrons and very-low-density lipoproteins (VLDL) (Wion et al., 1987; Dichek et al., 1991; Benlian et al., 1996; Su et al., 2004). Studies of Lpl^{-}/Lpl^{-} knock out mice have shown that LPL-deficiency causes severe hypertriglyceridemia, reduced high-density lipoprotein (HDL) levels and death within 18 h of birth (Weinstock et al., 1995). Human clinical studies have also examined loss of function *LPL* mutations leading to familial chylomicronemia or hyperlipoproteinemia type I, a rare recessive disorder appearing in children and characterized by dramatically reduced HDL-cholesterol ratios and very high blood triglyceride levels (Ameis et al., 1991; Faustinella et al., 1991; Mead et al., 2002; Merkel et al, 2002). In addition, human *LPL* polymorphisms influence significantly a number of major diseases, including atherosclerosis (Reymer et al., 1995; Shimo-Nakanishi et al., 2001; Tsutsumi, 2003; Stein and Stein, 2003), atherosclerotic cerebral infarction (Xu et al., 2008), ischemic stroke (Zhao et al., 2003), coronary artery disease (Zhang et al., 1998; Spence et al., 2003), pre-eclampsia (Hubel et al., 1999; Zhang et al., 2006), Alzheimer's disease (Papassotiropoulos et al., 2005; Blain et al., 2006), ulcerative colitis (Kosaka et al., 2006),

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hypertension (Chen et al., 2005), diabetes (Ukkola et al., 2005) and obesity (Huang et al., 2006; Radha et al., 2007).

Structures of several vertebrate *LPL* genes have been determined, including human (Wion et al., 1987; Chuat et al., 1992), mouse (Zechner et al., 1991), rat (Brault et al., 1992; The MGC Project Team, 2004) and chicken (Cooper et al., 1992). Several *LPL* cDNA and amino acid sequences have also been reported for other vertebrates including gorilla (*Gorilla gorilla*) and rhesus monkey (*Macaca mulatta*) (Martinez et al., 2001), baboon (*Papio anubis*) (Cole and Hixson, 1995), pig (*Sus scrofa*) (Harbitz et al., 1992), cow (*Bos taurus*) (Senda et al., 1987), sheep (*Ovis aries*) (Edwards et al., 1993), cat (*Felis catus*) (Ginzinger et al., 1996), goat (*Capra hercus*) (Badaoui et al., 2007) and guinea pig (*Cavia porcellus*) (Enerbaeck et al., 1987) and fish species, sea bass (*Dicentrarchus labrax*) (Jose Ibanez et al., 2008) and bream (*Sparus aurata; Pagrus major*) (Saere-Vila et al., 2005; Oku et al., 2006). *LPL* genes usually contain 9 exons of DNA encoding LPL sequences which may undergo exon shuffling generating several isoproteins in each case (Thierry-Mieg and Thierry-Mieg, 2006). Three dimensional studies of pancreatic lipase (LIPP) (Winkler et al., 1990; Bourne et al., 1994) and molecular modeling of human LPL (van Tilbeurgh et al., 1994) have enabled identification of three major structural domains for the mammalian neutral lipase family, including an N-terminal domain with a catalytic triad of serine, aspartate and histidine residues; a 'lid' domain which covers the active site and contributes to the specificity for triglyceride and phosphoglyceride substrates; and a C-terminal or 'plat' domain, which contributes to lipid binding and specificity. LPL is synthesized by the endoplasmic reticulum (ER) of parenchymal cells and sequentially processed by the Golgi and ER with the addition of carbohydrate (Ailhaud, 1990; Stins et al., 1993; Hata et al., 1993). LPL is also subject to proprotein convertase cleavage at a site in the 'hinge' region separating the N- and C-terminal enzyme domains (Jin et al., 2005) and behaves as a homodimer with a proposed head-to-tail conformation (Murthy et al., 1996; Wong et al., 1997; Kobayashi et al., 2002). Following secretion, LPL binds to heparan sulfate proteoglycans on the endothelial surface by electrostatic charge effects onto the luminal surface of capillary endothelial cells and macrophages (reviewed by Tsutsumi, 2003).

This paper reports the predicted gene structures and amino acid sequences for several vertebrate *LPL* genes and proteins, the predicted secondary and tertiary structures for vertebrate LIPL enzymes, several potential sites for regulating human *LPL* gene expression and the structural, phylogenetic and evolutionary relationships for these genes and enzymes with those for human, mouse and rat lipase gene families.

2. Methods

2.1. Vertebrate LPL gene and protein identification

BLAST (Basic Local Alignment Search Tool) studies were undertaken using web tools from the National Center for Biotechnology Information (NCBI) (http://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al., 1997). Protein BLAST analyses used vertebrate LPL amino acid sequences previously described (Table 1). Non-redundant protein sequence databases for several mammalian genomes were examined using the BLASTP algorithm, including human (*Homo sapiens*) (International Human Genome Sequencing Consortium, 2001); chimpanzee (*Pan troglodytes*) (Chimpanzee Genome Analysis Consortium, 2005); orang-utan (*Pongo abelii*) (http://genome.wustl.edu); cow (*B. taurus*) (Bovine Genome Project, 2008); horse (*Equus caballus*) (Horse Genome Project, 2008); mouse (*Mus musculus*) (Mouse Sequencing Consortium, 2002); rat (*Rattus norvegicus*) (Rat Genome Sequencing Consortium, 2004); opossum (*Monodelphis domestica)* (Mikkelsen et al., 2007); platypus *(Ornithorhynchus anatinus)* (Warren et al., 2008); frog (*Xenopus tropicalis*) (http://genome.jgi-psf.org/Xentr3/Xentr3.home.html); stickleback (http://www.broadinstitute.org/models/stickleback)

Table 1
Vertebrate lipoprotein lipase (*LPL*) genes and proteins. RefSeq: the reference amino acid sequence; [1,3]predicted Ensembl amino acid sequence; [2]not available; Contig refers to a DNA scaffold for sequencing analyses; GenBank IDs are derived NCBI http://www.ncbi.nlm.nih.gov/genbank/; Ensembl ID was derived from Ensembl genome database http://www.ensembl.org; UNIPROT refers to UniprotKB/Swiss-Prot IDs for individual acid lipases (see http://kr.expasy.org); bps refers to base pairs of nucleotide sequences; pI refers to theoretical isoelectric points; the number of coding exons are listed.

Lipoprotein lipase gene *LPL*	Species	RefSeq ID [1]Ensembl (predicted)	GenBank ID	UNIPROT ID	Amino acids	Chromosome location	Exons (strand)	Gene Size bps	pI	Subunit MW	Signal peptide (cleavage site)
Human	*Homo sapiens*	NM_000237.2	BC011353	P06858	475	8:19,841,232-19,864,008	9 (+ve)	22,777	8.4	53,163	1-27 [AA-AD]
Chimpanzee	*Pan troglodytes*	[1]XP_001149804.1	[2]	[2]	475	8:16,183,708-16,206,551	9 (+ve)	22,844	8.5	53,162	1-27 [AA-AD]
Orangutan	*Pongo abelii*	[3]	[2]	[2]	475	8:19,482,810-19,505,529	9 (+ve)	22,720	8.5	53,133	1-27 [AA-AD]
Rhesus	*Macaca mulatta*	[1]ENSMMUT00000006658	AF403770	Q95MH0	475	8:19,847,146-19,860,974	9 (+ve)	13,829	8.5	53,146	1-20 [TA-SR]
Baboon	*Papio anubis*	NP_001106082.1	U18091	P49060	475	[2]	[2]	[2]	8.5	53,146	1-20 [TA-SR]
Marmoset	*Callithrix jacchus*	[3]	[2]	[2]	475	Contig4830:148,445-170,321	9 (−ve)	21,877	8.5	53,165	1-27 [DA-AD]
Mouse	*Mus musculus*	NM_008509.2	BC003305	P11152	474	8:71,404,652-71,426,282	9 (+ve)	21,631	8.0	53,109	1-27 [AA-AD]
Rat	*Rattus norvegicus*	NP_036730.1	BC081836	Q06000	474	16:22,536,120-22,556,716	9 (−ve)	20,597	8.4	53,082	1-27 [AA-AD]
Guinea Pig	*Cavia porcellus*	[1]ENSCPOT00000004098	[2]	P11153	475	1:57,048,993-57,068,443	9 (+ve)	19,451	8.8	53,522	1-27 [AA-AK]
Horse	*Equus caballus*	[1]XP_001489627.1	[2]	[2]	468	2:49,071,398-49,090,148	9 (+ve)	18,751	9.0	52,467	1-21 [AA-DR]
Cow	*Bos taurus*	NP_001068588.1	[2]	[2]	478	8:70,187,336-70,209,826	9 (+ve)	22,491	8.8	53,378	1-23 [RG-GL]
Dog	*Canis familaris*	[1]XP_534584.2	[2]	[2]	471	25:40,075,103-40,095,543	9 (−ve)	20,441	8.5	52,559	1-21 [AA-AR]
Rabbit	*Oryctolagus cuniculus*	NM_001177330.1	FJ429312	[2]	474	15:4,554,425-4,578,617	9 (+ve)	24,193	8.2	52,977	1-20 [TA-SR]
Pig	*Sus scrofa*	[1]ENSSSCT00000010522	AK344311	P11151	478	14:3,826,571-3,852,602	9 (+ve)	26,032	8.6	53,498	1-26 [LA-TA]
Elephant	*Loxodonta africana*	[1]ENSLAFT00000005641	[2]	[2]	472	22:17,198,146-17,219,228	9 (+ve)	21,083	9.0	52,937	1-20 [PA-SH]
Opossum	*Monodelphis domestica*	[1]XP_001381955.1	[2]	[2]	478	1:580,795,573-580,818,319	9 (+ve)	22,747	8.6	53,362	1-21 [TS-TG]
Platypus	*Ornithorhynchus anatinus*	[1]ENSOANT00000009473	[2]	[2]	476	5:4,223,188-4,247,644	9 (−ve)	24,457	8.4	53,558	1-26 [AA-SD]
Chicken	*Gallus gallus*	NM_205282	AB016987	P11612	475	Z:53,400,437-53,408,327	9 (−ve)	7,891	8.5	53,636	1-25 [AG-SD]
Frog	*Xenopus tropicalis*	[1]ENSXETT00000056503	[2]	[2]	466	sc79:338,410-419,025	9 (−ve)	80,616	8.2	53,153	1-18 [AT-KL]
Stickleback	*Gasterosteus aculeatus*	[1]ENSGACT00000015067	[2]	[2]	514	VIII:14,407,768-14,412,555	10 (−ve)	4,788	8.2	58,052	1-23 [FS-SD]