Supplementary Table 1. AGPAT family

Human	C. elegans homologue
GPAT1	acl-6
GPAT2	acl-6
GPAT3	acl-4, acl-5
GPAT4	acl-4, acl-5
AGPAT1	acl-1, acl-2
AGPAT2	acl-1, acl-2
DHAPAT	acl-7
LYCAT/LCLAT1/ALCAT1	acl-8, acl-9, acl-10
LPGAT1	acl-12, acl-13, acl-14
AGPAT5	acl-11
Tafazzin	acl-3
AGPAT3	-
AGPAT4	-
LPCAT1	-
LPCAT2/LysoPAFAT	-
LPEAT2	-

acl-1~14; AGPAT family members in C. elegnas. At least 16 genes belong to the AGPAT family in human. AGPAT3, AGPAT4, LPCAT1, LPCAT2/LysoPAFAT and LPEAT2 are not conserved in C. elegans. GPAT, glycerol-3-phosphate acyltransferase; AGPAT, 1-acylglycerol-3-phosphate Oacyltransferase; DHAPAT, dihydroxyacetonephosphate acyltransferase; LYCAT and LCLAT, lysocardiolipin acyltransferase; ALCAT, acyl-CoA:lysocardiolipin acyltransferase; LPGAT, lysoPG acyltransferase; LPCAT, lysoPC acyltransferase; PAF, platelet-activating factor; LPEAT, lysoPE acyltransferase.

Supplementary Figure 1

Α

Rat Chicker	1						- M V - M V	SWI	CGI CGI	Y F I Y F I	L F		GS	FFO	isi isi isi	FML	GP GP		PLM	FI	NLSI	W Y R V W Y R V	1 S S 1 S S	44 44
Xenopus	1						- M V	SWI	RGV	YFV YFV	LA		GS	FFO	S I S I	FML	GP GP	FL	PLM	F I L V	S P A S P S	W Y RV W Y RV	V I T D V I T D	44
ACL-8	1						- M V		(GV (GV	TFI		VE	SS		TV	FLL	FP			WF	S P S A P K	RYRV LW <mark>R</mark> 1	TCAD	44 44
ACL-9 ACL-10	1	M S S L	- N E	P P V 	LGA -MR	GQQ	IGT	PLI	GW	FFG	i A A i L C	MLL	S A	FFO	IV L NY	YIV	(T P [L F		/ L L . P I	FLI	K P R R H K			60 50
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Mouse	45	RLV/	TW		PVA	LLE	TMF	GV	VV	ITC	i D A	FV	GE	RS	/ 1 1	MNH		RV	N W C	FLV	NC	LMR	Y S	102
Chicken	45	RIV	TW		PVA	LLE	MV	GAI	vv	V T C	DG	F I	GE	RS	/ 1 1	MNF		RM	D W M	FLV	N N C		Y S	102
Xenopus Zebrafish	45 45	RIVA	A T W	LTL	P V A P V A	LLE		GAI		ITC VTC	i D G i D G	F I F I	P G E P G E	RSV	/ 1 1 / 1 1	MNE	IR T IR T	RLC	M W C	FLV	NNC NCC		Y S Y S	102 102
ACL-8	45 61		B F W	LTF	PCS		WVF	GV		V T C	i D L	IEF				MNH			L W C	FS	N N A	YK		104 120
ACL-10	51	RAIS	SYW	MT 1	PMG	LLE	FLN	GV	RIR	vsc	DE	IEF	GS	PAI	v	MNH	IRT	RLC	W M	Υм	C A	YQ	INPW	110
					_							Mot	tif II					_			_			
Mouse Rat	103 103	YLRV	EK	ICL	K S S K S S	L K S L K S	VPG	FGV	V A M		AF		I H R I H R	KWI		K S H	1 F E 1 F E		IDY /DY	FC/ FC/	A I H A I H	E P L C E P L C	9 L L I 9 L L I	162 162
Chicken	103	YLRL	EK	ICL	KSS	LKS	1 P G	FGV	A M	QVA	AF	IF	QR	ĸw	DD	KSH	FE	NMI	HY	FC	DIH	EPL	9 L L I	162
Zebrafish	103	YLR	EK		KAA	LKS	VPG	FGV	V A M		SF	IF	QR	RW		RT	MS	NMI	QY	FC	RIR			162
ACL-8 ACL-9	105 121	LCTI	T E K	ISL ISL	K A P K G M	LKK	1 P G V P G	AGV	V A M V A M	SSG	isy sy	I F L I F L	DR DR	NF	N D D T D	К Р V К Т К	(L] (L]D	RIN	/ K Y N Y	YSO	GSE ETE	K K Y Y K Y	9 1 L L 9 L L L	164 180
ACL-10	111	LITS	S N K	ISL	KAQ	LKK	LPG	AGI	GM	AAA	QF	V F I	. E R	NA	VD	KRS	FD	DAI	DY	FK	N I D	ККҮ	9 I L L	170
Maura	162	M	otif										Mot	if I	V									222
Rat	163	FPEC FPEC	G T D G T D	L T E L T <u>E</u>	NNK	ARS	NDF	AER	(NG) (NG)		(YE (YE	YVI YVI	. НР . НР	RT	r G F F G F	TEV	V D	RLF	RER	RN	L D A	V H D V H D		222
Chicken Xenopus	163 163	FPEC	G T D G T D		N Т К N Т К	ARS	NDF	AE	K N G	LRK	YE	Y V I Y V I	. H P	RT	r G F	TEV			REG	N N N N		IHD IHD		222 222
Zebrafish	163	FPEO	GTD	LTE	NTR	ARS	DEF	AE	(N G	LOK	YE	ΥVΙ	HP	RT	GF	T F 1	V D	TLF	GG	DN	DA	ЧНD	ITVA	222
ACL-9	181	FPE	G T D	KCP	KAT	ERS	RIH	SEI	KG	LVH	I Y Q	YVI	. <u>н</u> Р	RV	GF	V H I	∎ v o	AM	RA	NN	IKY		VSIG	240
ACL-10	171	FPEO	G T D	KSE	νтт	LKS	REF	AK	(NG	L R H	ILD	ΥVΙ	Y P	RT	r G F	LHL	. L N	КМ	R E Q	EY	VEY	IYD		230
Mouse	223	YPYN	NIP	QTE	кнг	LLG	- D F	РК	ТН	EHV	OR	Y P/	A D S	LPI	r s k	EDI	QL	wc		WE	EKE	ERLE	RSFY	281
Rat	223	YPY	NIP	QTE	KHL	LLG	- D F	PK	ΙH	FHV	HR	YP	DT	LP	ΓSK	EDL	QL	WC	IKR	WE	EKE	ERLI	RSFY	281
Xenopus	223	YPUN		QTE	K H I		- N F	PKE	2 I H 2 I H	FHV	CR	YP	/ S S	LPN	ISK ISK	EEL	. Q L Q L	wcc		WK	EKE		RAFY	281
Zebrafish ACL-8	223 225	YPON		Q T E D T E	R H L A K L		- V F - N F	PRE	I H	FHV	Q R	FTN	/ A S . D E		- T G	AG	O A C E K	WC WL1		W R I W A 1	EKE	RRLO	ORFY KKFY	281 282
ACL-9	241	FGD/	A I V	QSE		FAH	GVO	PK	VF	YQ	IK	YP	EA	I P C	TD		GQ	WLV		WRI	KE	EKLI	K R F Y	300
ACLIN	201	1 2 1		000	10	VLN	GAB		V LU		RK		100			004	1 S R				IN E			200
Mouse	282	QGE		- K N	FHF	TGQ						- s 1	V P	PC	(SE	LRV	LV	VKL	. L <mark>S</mark>	IV	YWA	LFC	SAMC	323
Rat Chicken	282 282	QGE-		- К N - К С	FHF	T G Q T G Q						- S 1 - S 1	V P	PC	(SE (SE	LRV	LV	VKL	LLS	IL LL	YWA YWT	L F C S	SAMC NGTF	323 323
Xenopus	282	QGE		- R Y	FDA	TRR						- S F	R I P	PCI	(SE	LRV	ΗL	1 K	AAS	LL	ΥWΤ	LFPI	AMI	323
ACL-8	283	EQE		R		SGD	R					- F 6	wP	- E 1	TT	GIG	Y Y	VAF	AF	wv	LAS	<u>1</u> w1	M G A I	324
ACL-9 ACL-10	301 291	SEE	RNV 2PI	- RQ NRQ	F P D F P V	ERG	G D					- V E	EYE /WR	SW	N N T		Q K I F Y	G L 1 V K L	IGF TS	W C I	FTT FWT		M F M F S F C S	346 337
		_			_									-	_	_								
Mouse	324	LLIN	ĹY	SPV	RWY	F 1 1	S I V	FF	/ L Q	ERI	FG	GLE	11	EL/	C Y	RFL	нк	HPH	I L N	SKI	KNE		376	
Chicken	324	ALLI	ΥĽΫ́	SFA	RWY	FAA	MII	IFV		O K I	F G	GLE	LI	EL/	A C H		кк			DTI	KMK	кк-	378	
Xenopus Zebrafish	324 339	VLLY	IL Y	S P V P P A	R W Y Q F Y	F L V F L F	T V V M V V	I F N	/LQ _CQ	E K I Q R F	FG	GLE	LI LM	EL/ EL/	АСН АСН	RYY	TR SR	RYK	K R G	QE			371 388	
ACL-8	325 347	YSLL	WV	KIY	VSI	AII	FYL	ASI	RF		YN	GA	FΥ	FLF	RWF	EAR	RD	CDH	(TS	KKI	E		372 399	
ACL-10	338	YHIF	FV	RTL	QLG	FLY	FFV	1 5 1	YL	SWR	YG	GII	КΥ	11	F K W	QES	RK	SLO	ĸs	PS	SSS	1	391	

В

	Motif I	Motif II	Motif III	Motif IV
Mouse GPAT1	²²⁷ LPVHRSHIDYLL	²⁷³ GFFIRRR	³¹³ FLEGTRSRS	³⁴⁷ ILVIPVGISY
Mouse AGPAT1	⁹⁸ VS <mark>NH</mark> QSSLDLLG	¹⁴¹ II <mark>FIDR</mark> K	¹⁷³ FPEGTRNHN	²⁰⁰ VPII <mark>P</mark> IVMSS
Mouse LYCAT Rat LYCAT Chicken LYCAT Xenopus LYCAT Zebrafish LYCAT ACL-8 ACL-9 ACL-10	82IMNHRTRVDWMF 82IMNHRTRVDWMF 82IMNHRTRNDWMF 82IMNHRTRLDWMF 82IMNHRTRLDWLF 98IMNHRTRLDWLF 88VMNHRTRLDWWY	¹³⁰ FIFIHRK ¹³⁰ FIFIQRK ¹³⁰ FIFIQRK ¹³⁰ FIFIHRK ¹³⁰ FIFIQRR ¹³² YIFLDRN ¹⁴⁸ YIFLDRS ¹³⁸ FVFLERN	¹⁶³ FPEGTDLTE ¹⁶³ FPEGTDLTE ¹⁶³ FPEGTDLTA ¹⁶³ FPEGTDLTD ¹⁶³ FPEGTDLTE ¹⁶⁵ FAEGTDKGE ¹⁸¹ FPEGTDKSE	¹⁹¹ YVLHPRTTGF ¹⁹¹ YVLHPRTTGF ¹⁹¹ YVLHPRTTGF ¹⁹¹ YVLHPRTTGF ¹⁹¹ YVLHPRTTGF ¹⁹³ YVLHPRTTGF ²⁰⁹ YVLHPRVTGF ²⁰⁰ YVLYPRTTGF
LYCAT consensus	XMNHRTRXDWXF	XIFXXRX	FXEGTDXXX	YVLHPRTTGF

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Supplementary Figure 1. Alignment of the LYCAT/ACL-8, -9, -10 subfamily members in AGPAT family. (A) Alignment of *C. elegans* ACL-8, ACL-9, ACL-10 and the closest homologues (LYCAT) from mouse, rat, chicken, xenopus and zebrafish. Identical amino acids are shown on a black background and similar amino acids are on a grey background. The four conserved AGPAT motifs (motif I~IV) are boxed. Accession numbers for the sequences used were as follows: mouse: NP_001074540; rat: XP_343021, chicken: NP_001026210; xenopus: NP_001135517, zebrafish: NP_998435; ACL-9: NP_504644; ACL-10: NP_505971. The amino acid sequence of ACL-8 was determined as described previously (12). (B) Analysis of conserved amino acids of LYCAT proteins in the AGPAT motifs. Red amino acids show consensus motifs that define AGPAT family. Blue amino acids are "LYCAT signature amino acids", which are highly conserved in LYCAT proteins of various species, but not among other AGPAT family members, such as GPAT1 or AGPAT1. Numbers refer to amino acid residue position within each protein sequence. Accession number: mouse GPAT1; NP_032175, mouse AGPAT1; NP_001156851.

Supplementary Figure 2



Supplementary Figure 2. A scheme of the procedure for determining *sn*-2-acyl LPLAT activity.

After the *in vitro* acyltransferase assay, the lipids were extracted and separated by TLC as described in *Materials and Methods*. The resulting Phospholipid fractions were re-extracted from the TLC plates and treated with phospholipase A_2 . The distribution of radioactivity among the reaction products (free fatty acid and lysophospholipid) was assessed following TLC. X; polar head group, LPLAT; lysophospholipid acyltransferase, FA; fatty acid, FFA; free fatty acid.

Supplementary Figure 3



Supplementary Figure 3. Plasma and liver lipid levels, serum AST and ALT levels were not altered in LYCAT^{-/-} mice. Plasma triglyceride and total cholesterol (A), hepatic triglyceride (B), serum AST and ALT (C) levels in LYCAT^{+/+} and LYCAT^{-/-} mice were determined. LYCAT^{+/+}, closed bars; LYCAT^{-/-}, open bars. Data represent the mean \pm SEM of triplicate measurements.