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Genomic organization of the DGAT2/MOGAT gene family in cattle *(Bos taurus)* and other mammals

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Abstract. We report the cloning and initial characterization of the genes encoding DGAT2 (diacylglycerol transferase 2), MOGAT1 and MOGAT2 (monoacylglycerol transferases 1 and 2) in domestic cattle *(Bos taurus)*. The three closely related genes belong to a gene family with at least eight members in mammals and are candidate genes for quantitative traits related to dietary fat uptake, lipid synthesis and storage. MOGAT2 and DGAT2 form a tandem and were mapped to bovine chromosome (BTA) 15q25→q26 by fluorescence in situ hybridization. MOGAT1 was localized to BTA 2q43→q44. The three genes were investigated for polymorphisms that might be associated with breeding values for milk fat percentage in the dairy breeds German Holstein, German Simmental and German Brown. All the detected polymorphisms were located outside exons or, with one exception, were silent. In MOGAT1, a missense mutation in exon 4 was found that causes a non-conservative substitution of cysteine^{170} (uncharged, hydrophobic) by lysine (positively charged, hydrophilic). However, allele frequency estimates from pooled DNA samples revealed no significant association of the observed polymorphisms with breeding values for milk fat percentage. A comparative analysis of chromosomal locations and exon-intron structure of the known members of the DGAT2/MOGAT gene family in humans, rodents and cattle indicates an ancient tandem duplication of the ancestor gene combined with an intron gain (or loss) in one copy. Further members of the family may have arisen by duplications of this gene tandem via two rounds of interchromosomal or genome duplications as well as further local (single) gene duplication and loss events.

Triglycerides (triacylglycerols) are the major energy storage molecules in eukaryotes. The final, and presumably rate-limiting step of triglyceride synthesis is catalyzed by a diacylglycerol acyltransferase (DGAT) (Mayorek et al., 1989). DGAT1 was the first identified gene encoding a protein with DGAT activity (Cases et al., 1998). A missense mutation (Lys^{232}→Ala) in DGAT1 has been shown to be significantly associated with variation in milk fat percentage in cattle. DGAT1 is likely causal for a QTL near the centromere of bovine chromosome 14 (Grisart et al., 2002; Winter et al., 2002). Generation of viable DGAT1-knockout mice (Smith et al., 2000) revealed that DGAT-like activity is found in other enzymes encoded by other genes and led to the detection of DGAT2. In humans, DGAT2 is expressed in many tissues. Highest mRNA levels were found in the liver, white adipose tissue and the mammary gland (Cases et al., 2001).

DGAT2 was the first identified member of a gene family with at least eight members in mammals (Cases et al., 2001). To date, this family has not been fully characterized in any single mammalian species. As such, the nomenclature for the family has not been finalized. This is especially the case with those members encoding monoacylglycerol acytransferase activity. The latter are referred to as "MGATs" in the literature but have been designated provisionally as "MOGATs" by the Nomenclature Committee of the Human Genome Organization (HGNC; Wain et al., 2002) because the MGAT symbol is reserved for another gene family. We follow the usage of the HGNC herein.

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DGAT2 and its relatives encode intrinsic membrane proteins that are completely unrelated to DGAT1. Hydrophobic analysis of the respective amino acid sequences reveals nine putative transmembrane domains in human DGAT1, but only two in human DGAT2 (Oelkers et al., 1998; Cases et al., 2001). Recently, three members of this gene family have been characterized in mice: MOGAT1 (Yen et al., 2002), MOGAT2 (Cao et al., 2003; Yen and Farese, 2003) and MOGAT3 (Cheng et al., 2003). MOGAT enzymes catalyze the synthesis of triglycerides from 2-monoacylglycerols and acyl-CoA. The so-called monoacylglycerol pathway is essential for intestinal dietary fat resorption. Here we report the cloning, physical mapping and sequence analysis of DGAT2, MOGAT1 and MOGAT2 in cattle *(Bos taurus)*. Additionally, we screened for intragenic polymorphisms and performed an initial association study of the three genes with milk fat percentage in the three dairy breeds German Holstein, German Simmental and German Brown.

Materials and methods

Isolation of genomic clones

Human nucleotide sequences (GenBank accession nos. DGAT2, BC015234; MOGAT1, AF384163; MOGAT2, AK026297; DGAT2L3, XM_088691; DGAT2L4, XM_088683) and BLAST algorithms (Altschul et al., 1990) with translated nucleotide sequences (TBLASTX) were used to search the expressed sequence tags database (dbEST) of GenBank (Boguski et al., 1993). EST sequences were found for bovine DGAT2 (GenBank accession nos. BE724193, BI536057, AW326247, BI681948, BE482224, BE479873, BF868335, BG694175, BG687855 and BF430191) and bovine MOGAT1 (GenBank accession nos. AW429404 and BE754760). The ten EST sequences for DGAT2 represent the complete mRNA sequence from exons 1–8. The consensus sequence of the two ESTs for MOGAT1 covers exons 1–3 and 6. Bovine ESTs were assembled into consensus mRNA sequences. Human and mouse sequence data (sources: NCBI, http://www.ncbi.nlm.nih.gov/ and Ensembl, http://www.ensembl.org/) were used to obtain putative splice sites. PCR primers were designed using Primer3 software (Rozen and Skaletsky, 1998) and considering splice sites and intron sizes in humans. PCR primers for MOGAT1 exons 4 and 5 were derived from the human cDNA sequence (GenBank accession no. AF384163). Initial PCR primers for MOGAT2 were derived from a porcine MOGAT2 EST sequence (GenBank accession no. BE030672) spanning exons 1–4. The following primers and amplification products were used to screen the gridded bovine BAC library RPCI-42 (Warren et al., 2000):

DGAT2: 807 bp fragment (exon 5 – exon 6)
forward 5′-CAGGAACTACATCTTTGGGTACCA-3′
reverse 5′-ATTGCCACTCCCATTCTTTG-3′
MOGAT1: 347 bp fragment (intron 5 – exon 6)
forward 5′-ACAATCCAGCATGTGCAGAG-3′
reverse 5′-CTGGAATACCATACTTCCCTTTG-3′
MOGAT2: 422 bp fragment (exon 3 – exon 4)
forward 5′-CCCCCATCTGATGATGCT-3′
reverse 5′-TGCTCAGGATGTGAGCAGC-3′

BAC DNA was prepared using QIAGEN Plasmid Midi Kit (Qiagen, Hilden, Germany). The specificity of the isolated BAC clones was confirmed by PCR amplification and sequencing of the respective DNA fragments.

Fluorescence in situ hybridization (FISH)

Purified BAC DNA from clones RPCI42-269A1 (DGAT2 and MOGAT2) and RPCI42-307A24 (MOGAT1) was labeled with digoxigenin-dUTP by standard nick translation and hybridized with 10× excess of bovine Cot1-DNA to normal male bovine metaphase spreads. Probe hybridization was detected with monoclonal mouse-anti digoxigenin (Roche, Mannheim, Germany) and sheep-anti-mouse-FITC antiserum (Sigma-Aldrich, Deisenhofen, Germany). The chromosomal gene locations were assessed according to the standardized karyotype of domestic cattle *Bos taurus* (ISCNDC, 2000) by measuring the relative fractional length from the long arm telomere to the hybridization signal (Fl_{qter}) and by comparison with the G-band-like DAPI staining pattern. Chromosome measurements were made using the software program MicroMeasure (Reeves and Tear, 2000).

Genomic DNA sequencing and long-range PCR

BAC clones RPCI42-5L16 (DGAT2), RPCI42-362M12 (MOGAT1) and RPCI42-20B12 (MOGAT2) were used for direct sequencing using an ABI 377 automated sequencer and BigDye kit v2.0 (Applied Biosystems Division, Foster City, CA, USA). To assess the size of the larger introns, 20 ng of purified BAC DNA was amplified by long range PCR in 20 µl reactions containing 2 units of AmpliTaq Polymerase (Qiagen, Hilden, Germany), 0.1 unit of ProofStart DNA Polymerase (Qiagen, Hilden, Germany), 1× of AmpliTaq PCR buffer, 1.5 mM of $MgCl_2$, 300 µM of each nucleotide, 0.5 µM each of forward and reverse primer, 4 µl of Qiagen Q-solution and 2% DMSO. The PCR profile included 2 min at 95 °C, 35 cycles of 10 s at 94 °C, 1 min at 61 °C and 20 min at 68 °C.

Polymorphism analysis

Semen samples were obtained from bulls of the dairy breeds German Holstein (2 × 32 animals), German Simmental (2 × 32) and German Brown (2 × 20). To search for gene variants associated with milk fat percentage in each breed, bulls with extreme high (+) and low (–) breeding values for milk fat percentage were selected. Equal amounts of individual DNA-samples were pooled as described previously to minimize effort (Winter et al., 2002). Screening for polymorphisms in exons and smaller introns was done by resequencing the six pooled DNA samples as well as 12 individual DNA samples (German Holstein and German Simmental only).

Resequencing used a 20 µl PCR reaction containing 50 ng of genomic DNA, 0.5 units of HotStar Taq Polymerase (Qiagen, Hilden, Germany), 1× PCR buffer, 1.5 mM of $MgCl_2$, 200 µM of each nucleotide and 0.5 µM of each primer. The cycling profile was initial denaturation for 5 min at 95 °C; 35 cycles of 1 min at 94 °C, 1 min at 60 °C and 1 min at 72 °C; and final elongation of 3 min at 72 °C. PCR products were purified using MultiScreen-PCR filtration plates (Millipore, Eschborn, Germany). The purified PCR products were sequenced using an ABI 377 automated sequencer (see above). Sequence data were analyzed using the Phred/Phrap/Polyphred/Consed software suite (Nickerson et al., 1997; Ewing and Green, 1998; Ewing et al., 1998; Gordon et al., 1998). Allele frequencies were estimated by analyzing sequence traces from pooled DNA. For each polymorphism, normalized amplitude values of the two alternative bases from the pooled DNA were compared with their normalized amplitude values from homozygous and heterozygous individuals as described previously (Winter et al., 2002). The analysis was automated using Python scripts (available from authors upon request).

Results and discussion

A BAC clone containing both DGAT2 and MOGAT2 was mapped to bovine chromosome (BTA) 15q25→q26 (Fig. 1A). This agrees with the observation that DGAT2 and MOGAT2 also form a tandem some 40 kb apart in both humans and mice. MOGAT1 was assigned to BTA 2q43→q44 (Fig. 1B). FISH results and comparative mapping data are summarized in Table 1. In humans, mice and rats, eight members of the DGAT2/MOGAT gene family have been found, located on four different chromosomes. Together with cattle, all known family members in these species are located at the known respective orthologous chromosome segments. Current homology maps between any of humans, mice, rats and cattle (MGI: Mouse Genome Informatics, http://www.informatics.jax.org/, May 2003; Frönicke and Wienberg, 2001) allowed us to predict the chromosomal position of as yet unidentified DGAT members in cattle (e.g. on chromosome X).

Sequences of smaller introns and all exons except the last exon of MOGAT2 have been deposited in GenBank under the

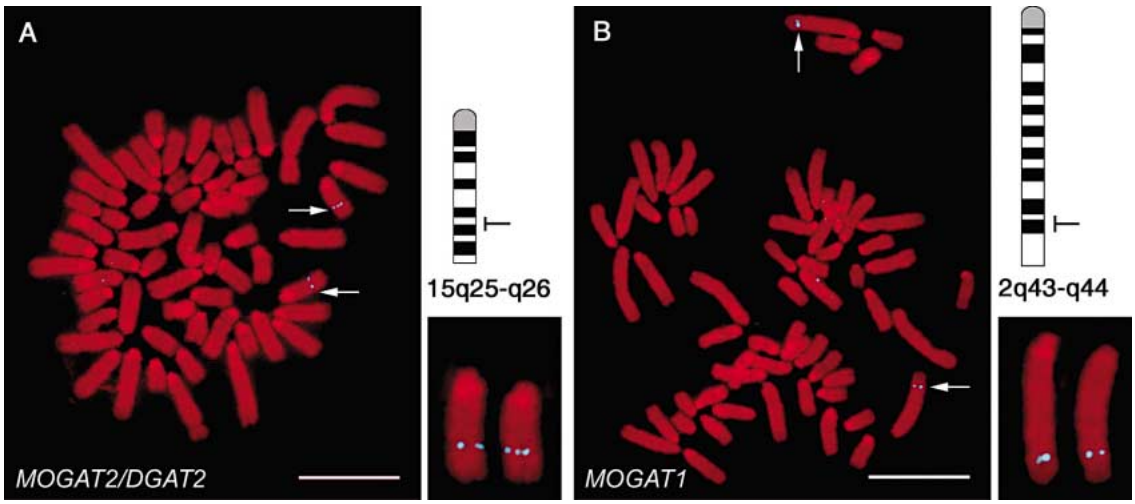

Fig. 1. Physical localization of DGAT2 and MOGAT2 (**A**) and MOGAT1 (**B**) in domestic cattle *(Bos taurus)* by fluorescence in situ hybridization. BAC clones containing the respective genes were hybridized to metaphase spreads from a normal bull. Chromosomes are counterstained with DAPI (pseudo-colored in red). Scale bar = 10 μm.

Table 1. Chromosomal locations of DGAT2/MGAT gene family members

Gene	n	L_{qter}[a] ± SD	Chromosomal position[b]			
			Cattle	Human	Mouse	Rat
MOGAT1	21	0.17 ± 0.03	2q43–q44 AJ519785[c]	2q36.2 BN000154[c]	1C4 AF384162[d]	9q33 XM_237315[c]
MOGAT2	22	0.27 ± 0.04	15q25–q26 AJ519786[c]	11q13.5 AY157608[d]	7E1 AY157609[d]	1q32 XM_218952[c]
DGAT2			15q25–q26 AJ519787[c]	11q13.5 BC015234[d]	7E1 AF384160[d]	1q32 AJ487787[d]
MOGAT3			25?	7q22 AY229854[d]	5G1 AC079872[e]	12q12 RNOR01027916[e]
DGAT2L7			25?	7q22 BN000168[c]	5G1 AC079872[e]	12q12 XM_222084[c]
DGAT2L3			X?	Xq12 BN000155[c]	XC3 XM_141972[c]	Xq21 XM_228568[c]
DGAT2L4			X?	Xq12 BN000156[c]	XC3 XM_141969[c]	Xq21 XM_228583[c]
DGAT2L6			X?	Xq12 BN000157[c]	XC3 XM_141971[c]	X?

[a] L_{qter} = relative fractional length from the long arm telomere to the signal position ± standard deviation.
[b] Chromosomal positions in humans, mice and rats were derived from genome draft sequences (http://www.ncbi.nlm.nih.gov/; http://www.ensembl.org). Question marks indicate putative gene family members and their chromosomal position as predicted from current homology maps (MGI: Mouse Genome Informatics, The Jackson Laboratory, Bar Harbor, Maine, http://www.informatics.jax.org, May 2003; Frönicke and Wienberg, 2001).
[c] Accession number refers to predicted mRNA.
[d] Accession number refers to cDNA.
[e] Accession number refers to genomic DNA.

following accession numbers: DGAT2 mRNA: AJ519787; DGAT2 gDNA: AJ534368, AJ534369, AJ534370, AJ534371, AJ534372; MOGAT1 mRNA: AJ519785; MOGAT1 gDNA: AJ534373, AJ534374, AJ534375, AJ534376; $MOGAT2^{trunc}$: AJ519786; MOGAT2 gDNA: AJ534377, AJ534378, AJ534379. Compared to the respective human sequence, bovine DGAT2, MOGAT1 and MOGAT2 show 90.2%, 84.1% and 80.2% identity of the coding sequence.

The gene polymorphisms that were identified by an initial screening in the dairy breeds German Holstein, German Simmental and German Brown are shown in Table 2. Resequencing of DGAT2 revealed 22 SNPs (single nucleotide polymorphisms) and a six-nucleotide insertion, all in untranslated regions. In intron 6 we found 12 linked SNPs (ID 293–304); individual sequencing of 38 animals showed that all were either homozygous for one of two haplotypes or heterozygous for all 12 SNP loci indicating the existence of only two haplotypes. In MOGAT1, we found three intron SNPs and a missense mutation (SNP ID 347) in exon 4 that leads to a non-conservative substitution of $cysteine^{170}$ (uncharged, hydrophobic) by lysine

Table 2. Polymorphisms in bovine DGAT2, MOGAT1 and MOGAT2

DGAT2				MOGAT1				MOGAT2			
SNP ID[a]	Position	Allele		SNP ID[a]	Position	Allele		SNP ID[a]	Position	Allele	
		1	2			1	2			1	2
intron 4 (AJ534371)[b]				intron 1 (AJ534374)				5' upstream (AJ534377)			
338	433	A	G	346	154	G	del	350	109	G	A
								351	156	G	C
intron 5 (AJ534371)				exon 4 (AJ534374)				352	361	G	A
291	755	A	G	347	426	G	C	353	421	A	C
-	959-66	ins[c]	T	Cys^{170} - Ser				354	463	C	T
292	1004	A	G					355	467	G	C
				intron 5 (AJ534374)				356	513	G	C
intron 6 (AJ534371)				348	1485						
293	1501	G	T			C	T	5'UTR (AJ534377)		G	A
294	1514	T	C	intron 5 (AJ534376)				357	578		
295	1541	C	G	349	502						
296	1578	C	T			C	T	exon 1 (AJ534377)			
297	1614	A	G					358	618	G	A
298	1637	A	G					silent			
299	1694	A	C								
300	1740	C	T					intron 1 (AJ534377)			
301	1766	A	del					359	724	C	T
302	1927	G	A					360	727	C	A
303	2012	T	G					361	757	C	T
304	2065	T	C					362	760	T	C
intron 7 (AJ534372)								exon 2 (AJ534378)			
339	349	A	G					363	290	G	A
340	357	A	G					silent			
341	396	A	G								
342	448	A	G					intron 4 (AJ534379)			
343	481	A	G					365	1188	A	G
344	668	C	G					366	1212	C	A
3'UTR (AJ534372)								intron 5 (AJ534379)			
345	975	A	G					367	1725	T	C

[a] SNP ID refers to the SNPZoo database, freely accessible via http://www.snpzoo.de/ (Fries and Durstewitz, 2001).
[b] GenBank database accession numbers are indicated in brackets.
[c] ins = CCCTGGCA.

(positively charged, hydrophilic). In MOGAT2, we found 15 SNPs outside exons and two silent exon SNPs (ID 358 and 363). A standard chi-square test did not reveal a significant association of allele frequencies with breeding values for milk fat content in any of the three analyzed dairy breeds (data not shown). However, the upstream regulatory regions have not yet been analyzed.

The exon-intron structure of bovine DGAT2, MOGAT1 and MOGAT2 is shown in the upper part of Fig. 2. All splice sites follow the GT–AG rule (Breathnach et al., 1978). The exon-intron borders of bovine DGAT2, MOGAT1 and MOGAT2 are completely conserved with respect to their human orthologues with the single exception that exon 1 of DGAT2 in cattle consists of only the first 40 bp of the corresponding exon in humans. Human MOGAT2 has a truncated splice variant that is terminated by a stop codon in intron 4 and encodes a protein without MOGAT activity (Yen and Farese, 2003). Bovine MOGAT2 also contains a stop codon in intron 4 suggesting that a similar splice variant may exist in cattle. Exon 6 of bovine MOGAT2 has not yet been sequenced.

With few exceptions, gene structure is conserved extensively within the DGAT2/MOGAT gene family as shown in the lower part of Fig. 2. An additional intron splits exon 2 of MOGAT1 and MOGAT2 into two exons in all other family members. This structural difference divides the gene family into a DGAT2 lineage (DGAT2, MOGAT3, DGAT2L3, DGAT2L4, DGAT2L6) and an MOGAT lineage (MOGAT1, MOGAT2, DGAT2L7). DGAT2 has also acquired an additional (first) exon at the 5′ end that has no equivalent in the other family members. The three X-chromosome family members (DGAT2L3, DGAT2L4, DGAT2L6), whose function is still unknown, show conservation of the last six exons compared with DGAT2 and MOGAT3. We are currently investigating chromosomal positions, gene structure changes and coding sequences in additional distantly related mammals to get more insight into the evolution of the gene family. Preliminary results indicate that the family originated with an ancient tandem duplication of the ancestral gene combined with an intron gain (or loss) in one copy. This event possibly predated vertebrates. One gene copy gave rise to the MOGAT lineage, the other to the DGAT2 lineage (see above). This gene tandem was

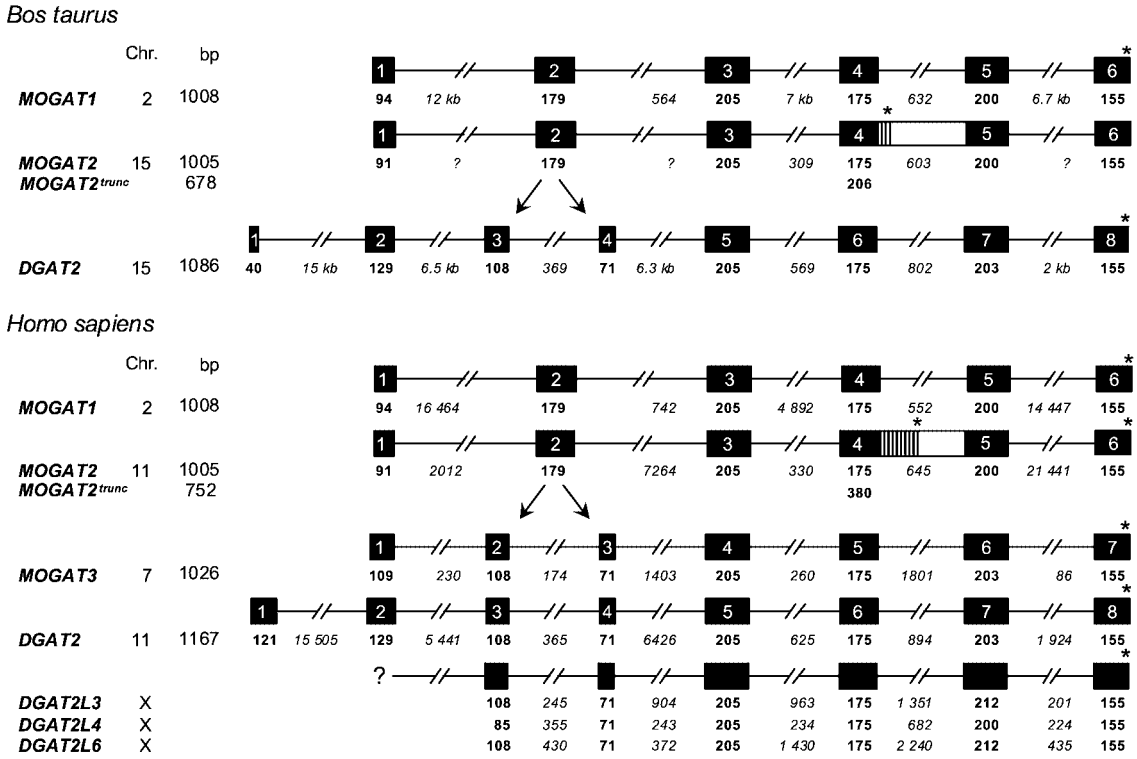

Fig. 2. Predicted exon-intron structure of DGAT/MOGAT gene family members in cattle (upper part) and humans (lower part). Exons are represented by black boxes and numbered in white. Exon and intron sizes are indicated in bp (or kb). The automatically predicted Ensemble exon-intron structure (http://www.ensemble.org) was manually corrected (Ensembl Accession numbers: MOGAT1, ENST00000264412; MOGAT3, ENSG00000106384; DGAT2L7, ENST00000323003; MOGAT2, ENSG00000166391; DGAT2, ENSG00000062282; DGAT2L3, ENSG00000180526; DGATL4, ENSG00000147160; DGATL5, ENSESTG00000023617). Human MOGAT2 has a truncated splice variant that is terminated by a stop codon in intron 4 (white box). Bovine MOGAT2 also contains a stop codon in intron 4 suggesting that a similar splice variant may exist in cattle. Exon 6 of bovine MOGAT2 has not yet been sequenced.

subject to subsequent interchromosomal duplications or genome duplications (tetraploidizations). In recent mammals, at least two such mixed gene tandems are still present: MOGAT2/DGAT2 (on human Chr 11, mouse Chr 7, rat Chr 1 and cattle Chr 15) and DGAT2L7/MOGAT3 (on human Chr 7, mouse Chr 5, rat Chr 12; for details, see Table 1).

The evolutionarily conserved tandem arrangement of a MOGAT and a DGAT gene might facilitate a concerted regulation of transcription (e.g. by a common upstream regulatory region). This would make sense because MOGAT provides the substrate for DGAT2. The three DGAT2 lineage members on the X chromosome likely resulted from local duplication events.

Information regarding the actual physiological role of DGAT2 and the closely related MOGAT genes in vivo is only just becoming available. High mRNA expression in liver tissue indicates that DGAT2 could be especially important for the assembly of fatty acids synthesized de novo from excess carbohydrates into VLDL lipoproteins. High expression of DGAT2 in adipose tissue also suggests a significant function in triglyceride storage (Cases et al., 2001). Recent experiments in mice revealed that DGAT2 mRNA expression is stimulated by insulin (Meegalla et al., 2002). MOGAT1, MOGAT2 and MOGAT3 are characterized by distinct tissue-specific expression patterns and substrate specificity of the encoded enzyme. MOGAT1 mRNA expression was found in adipose tissue, stomach and kidney from mouse, but not in the small intestine (Yen et al., 2002). MOGAT2 and MOGAT3 are both highly expressed in the intestine and could play an essential role in intestinal dietary fat absorption by re-synthesizing triglycerides from fatty acids and monoacylglycerol. MOGAT2, in parallel to DGAT2, is also highly expressed in the liver in humans (Yen and Farese, 2003). Recent in vitro experiments have shown that MOGAT3 also has a significant DGAT activity (Cheng et al., 2003). Expression of MOGAT3 was reported to be restricted to the gastrointestinal tract with the highest level found in the ileum (Cheng et al., 2003).

All members of the DGAT2/MOGAT gene family are high priority candidate genes for quantitative traits related to dietary fat uptake and triglyceride synthesis and storage in farm animals. Moreover, this gene family may also play a key role in polygenic diseases in humans such as obesity and type 2 diabetes. Blocking DGAT2/MOGAT enzymes in enterocytes might prove to be a feasible pharmaceutical target to inhibit intestinal fat absorption and therefore to treat obesity in humans (Yen and Farese, 2003).

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ: Basic local alignment search tool. J molec Biol 215:403–410 (1990).

Boguski MS, Lowe TM, Tolstoshev CM: dbEST database for "expressed sequence tags". Nature Genet 4:332–333 (1993).

Breathnach R, Benoist C, O'Hare K, Gannon F, Chambon P: Ovalbumin gene: evidence for a leader sequence in mRNA and DNA sequences at the exon-intron boundaries. Proc natl Acad Sci, USA 75:4853–4857 (1978).

Cao J, Lockwood J, Burn P, Shi Y: Cloning and functional characterization of a mouse intestinal acyl-CoA:monoacylglycerol acyltransferase, MGAT2. J biol Chem 278:13860–13866 (2003).

Cases S, Smith SJ, Zheng YW, Myers HM, Lear SR, Sande E, Novak S, Collins C, Welch CB, Lusis AJ, Erickson SK, Farese RV Jr: Identification of a gene encoding an acyl CoA:diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. Proc natl Acad Sci, USA 95:13018–13023 (1998).

Cases S, Stone SJ, Zhou P, Yen E, Tow B, Lardizabal KD, Voelker T, Farese RV Jr: Cloning of DGAT2, a second mammalian diacylglycerol acyltransferase, and related family members. J biol Chem 276:38870–38876 (2001).

Cheng D, Nelson TC, Chen J, Walker SG, Wardwell-Swanson J, Meegalla R, Taub R, Billheimer JT, Ramaker M, Feder JN: Identification of acyl coenzyme A:monoacylglycerol acyltransferase 3, an intestinal specific enzyme implicated in dietary fat absorption. J biol Chem 278:13611–13614 (2003).

Ewing B, Green P: Base-calling of automated sequencer traces using phred. II. Error probabilities. Genome Res 8:186–194 (1998).

Ewing B, Hillier L, Wendl MC, Green P: Base-calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Res 8:175–185 (1998).

Fries R, Durstewitz G: Digital DNA signatures for animal tagging. Nature Biotechnol 19:508 (2001).

Frönicke L, Wienberg J: Comparative chromosome painting defines the high rate of karyotype changes between pigs and bovids. Mammal Genome 12:442–449 (2001).

Gordon D, Abajian C, Green P: Consed: a graphical tool for sequence finishing. Genome Res 8:195–202 (1998).

Grisart B, Coppieters W, Farnir F, Karim L, Ford C, Berzi P, Cambisano N, Mni M, Reid S, Simon P, Spelman R, Georges M, Snell R: Positional candidate cloning of a QTL in dairy cattle: identification of a missense mutation in the bovine DGAT1 gene with major effect on milk yield and composition. Genome Res 12:222–231 (2002).

ISCNDB (2000): International System for Chromosome Nomenclature of Domestic Bovids, Di Berardino D, Di Meo GP, Gallagher DS, Hayes H, Iannuzzi L (coordinator) (eds). Cytogenet Cell Genet 92:283–299 (2001).

Mayorek N, Grinstein I, Bar-Tana J: Triacylglycerol synthesis in cultured rat hepatocytes. The rate-limiting role of diacylglycerol acyltransferase. Eur J Biochem 182:395–400 (1989).

Meegalla RL, Billheimer JT, Cheng D: Concerted elevation of acyl-coenzyme A:diacylglycerol acyltransferase (DGAT) activity through independent stimulation of mRNA expression of DGAT1 and DGAT2 by carbohydrate and insulin. Biochem biophys Res Commun 298:317–323 (2002).

Nickerson DA, Tobe VO, Taylor SL: PolyPhred: automating the detection and genotyping of single nucleotide substitutions using fluorescence-based resequencing. Nucl Acids Res 25:2745–2751 (1997).

Oelkers P, Behari A, Cromley D, Billheimer JT, Sturley SL: Characterization of two human genes encoding acyl coenzyme A:cholesterol acyltransferase-related enzymes. J biol Chem 273:26765–26771 (1998).

Reeves A, Tear J: MicroMeasure for Windows, version 3.3. (Free program, distributed by the authors via http://www.colostate.edu/Depts/Biology/MicroMeasure, 2000).

Rozen S, Skaletsky HJ: Primer3. Code available at http://www-genome.wi.mit.edu/genome_software/other/primer3.htm (1998).

Smith SJ, Cases S, Jensen DR, Chen HC, Sande E, Tow B, Sanan DA, Raber J, Eckel RH, Farese RV Jr: Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking *Dgat*. Nature Genet 25:87–90 (2000).

Wain HM, Lush M, Ducluzeau F, Povey S: Genew: the Human Gene Nomenclature Database. Nucl Acids Res 30:169–171 (2002).

Warren W, Smith TP, Rexroad CE 3rd, Fahrenkrug SC, Allison T, Shu CL, Catanese J, de Jong PJ: Construction and characterization of a new bovine bacterial artificial chromosome library with 10 genome-equivalent coverage. Mammal Genome 11:662–663 (2000).

Winter A, Kramer W, Werner FA, Kollers S, Kata S, Durstewitz G, Buitkamp J, Womack JE, Thaller G, Fries R: Association of a lysine-232/alanine polymorphism in a bovine gene encoding acyl-CoA:diacylglycerol acyltransferase (DGAT1) with variation at a quantitative trait locus for milk fat content. Proc natl Acad Sci, USA 20:20 (2002).

Yen CL, Stone SJ, Cases S, Zhou P, Farese RV Jr: Identification of a gene encoding MGAT1, a monoacylglycerol acyltransferase. Proc natl Acad Sci, USA 99:8512–8517 (2002).

Yen CL, Farese RV Jr: MGAT2, a monoacylglycerol acyltransferase expressed in the small intestine. J biol Chem 278:18532–18537 (2003).