Chicken Lecithin-Cholesterol Acyltransferase

MOLECULAR CHARACTERIZATION REVEALS UNUSUAL STRUCTURE AND EXPRESSION PATTERN*

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Rapidly growing oocytes in the laying hen are, in addition to the liver, targets of the so-called "reverse cholesterol transport" (RCT) (Vieira, P. M., Vieira, A. V., Sanders, E. J., Steyer, E., Nimpf, J., and Schneider, W. J. (1995) J. Lipid Res. 36, 601-610), pointing to the importance of this process in nonplacental reproduction. We have begun to delineate the details of this unique transport pathway by branch molecular characterization of the first nonmammalian lecithin-cholesterol acyltransferase (LCAT), the enzyme that catalyzes an early step in RCT. The biological significance of the enzyme is underscored by the high degree of protein sequence identity (73%) maintained from chicken to man. Interestingly, the conservation extends much less to the cysteine residues; in fact, two of the cysteines thought to be important in mammalian enzymes (residues 31 and 184 in man) are absent from the chicken enzyme, providing proof of their dispensability for enzymatic activity. Antibodies prepared against a chicken LCAT fusion protein cross-react with human LCAT and identify a 64-kDa protein present in enzymatically active fractions obtained by hydrophobic chromatography of chicken serum. The developmental and tissue distribution pattern of LCAT in females is striking: during embryogenesis and adolescence, LCAT expression is extremely high in liver, and LCAT transcripts are maintained at levels 5 times higher than in liver, in stark contrast to most mammals. In adult roosters, the levels of LCAT transcripts in brain are lower than in liver, and LCAT expression is maintained by hydrophobic chromatography of chicken serum. The developmental and tissue distribution pattern of LCAT in females is striking: during embryogenesis and adolescence, LCAT expression is extremely high in liver, and LCAT transcripts are maintained at levels 5 times higher than in liver, in stark contrast to most mammals. In adult roosters, the levels of LCAT transcripts in brain are lower than in liver, and LCAT expression is maintained at levels 5 times higher than in liver, in stark contrast to most mammals. In adult roosters, the levels of LCAT transcripts in brain are lower than in liver.

Together with the molecular characterization of chicken LCAT, these newly discovered developmental changes and gender differences in its expression establish the avian oocyte/liver system as a powerful model to delineate in vivo regulatory elements of RCT.

Reverse cholesterol transport (RCT)† is the physiological process that transports excess cholesterol back to the liver for secretion (1), thereby counteracting the accumulation of cholesterol in peripheral cells. The enzyme lecithin-cholesterol acyltransferase (LCAT, EC 2.3.1.43) catalyzes the initial step in this cascade, i.e. the esterification of cell-derived free cholesterol, concomitant with transfer of the esters into the core of high density lipoprotein (HDL) particles. Apolipoprotein A-I (apo-A-I), the major protein component of plasma HDL, is a potent activator of LCAT (2). By a yet poorly understood mechanism, the cholesteryl esters carried in HDL are then transported via the plasma back to the liver. The important physiological role of this enzyme has become evident from studies of mutations in the LCAT gene causing functional deficiencies of the enzyme (3). Patients with LCAT deficiency accumulate unesterified cholesterol in their peripheral tissues, associated with premature atherosclerosis, kidney disease, and central nervous system impairment (4, 5).

In mammals, the liver is the sole physiologically important target for RCT-derived peripheral cholesterol. However, in the course of our studies of lipoprotein metabolism in the chicken, we recently have identified an alternative deposition site, i.e. the developing oocytes of laying hens (6). In agreement with these findings, laying hens have been reported to possess high RCT activity and plasma HDL levels (7, 8). Results from Smith and co-workers (9) imply that the majority of the cholesteryl esters in chicken plasma are synthesized by LCAT. High plasma levels of HDL possibly reflect the significant demand for yolk lipids in the growing oocytes, single cells that accumulate as much as 5 g of lipid in the form of plasma-borne lipoproteins. It seems likely that supplementation of the oocytes with lipoproteins cannot be satisfied merely by uptake of very low density lipoprotein (VLDL) and vitellogenin. Indeed, the oocyte appears to compete with the liver for the products of the RCT; recently, we have identified bona fide, cholesteryl ester-containing HDL particles in the yolk of chicken oocytes (6). Thus, in the chicken, and possibly in other oviparous species, RCT and LCAT are tightly linked to the reproductive effort. Despite this obvious biological significance, knowledge about molecular characteristics of LCAT in any nonmammalian species is lacking.

In this paper we report the molecular characterization of LCAT from Gallus domesticus. Remarkable is the low degree of conservation of cysteine residues in the otherwise highly conserved, catalytically active, avian enzyme. The current studies also have revealed a hitherto unrecognized striking developmental and sex-specific pattern of LCAT gene expression in this avian species, i.e. a sharp rise of LCAT transcript levels in the brain of sexually maturing hens.

MATERIALS AND METHODS

Amplification of Chicken LCAT cDNA by PCR—cDNA from a chicken liver gt11 cDNA library (Clontech, Palo Alto, CA) was isolated accord-

base pair(s); PCR, polymerase chain reaction.
ing to standard procedures (10). A fragment with the size of 828 bp was amplified by PCR using the following degenerated primers derived from the human LCAT sequence: 5'-GGGATT (CTT) (CTT) (ATT) (A/ C) TGG-3' and 5'-CCACTACA(C) ACCCXXX(T) (CTC) (CTC) (AA) (A/ A)-3' (where X is any of the four bases), respectively. The PCR conditions were as follows: 1 min at 94°C, 1 min at 42°C, 2 min at 72°C, 30 cycles. The PCR fragment (828 bp) was subcloned into the pGEM-T vector system (Promega, Madison, WI), identified as chicken LCAT-derived cDNA by DNA sequencing, and used for library screening.

Screening and Clone Analysis—Two million independent plaques from a chicken embryo agt11 cDNA library were screened in duplicate. The library was obtained from whole 10-day-old embryos (Clontech, Palo Alto, CA). Clones were transferred to Hybond N+ membrane and immobilized by UV cross-linking. Filters were hybridized in 1% bovine serum albumin, 50% formamide, 100 mM sodium phosphate buffer, pH 9.5, incubated with 0.1% SDS at 42°C for 30 min, washed in 2× SSC, 0.1% SDS at 65°C. Phage DNA was purified from four strongly hybridizing plaques. The cDNA insert (1929 bp) was amplified by PCR with the help of agt11-specific primers (Clontech, forward primer 1218 and reverse primer 1222). The PCR conditions were as follows: 1 min at 94°C, 1 min at 60°C, 2 min at 72°C, 30 cycles. The fragment resulting from subcloning into the pGEM-T vector system, and two clones were sequenced on both strands using the diodeoxy chain termination method.

Northern Blot Analysis—For Northern blot analysis, 15 mg of total RNA prepared by the Trisolv method (Biotecx, Houston, TX), was denatured in 1× deionized glyoxal, 48% dimethyl sulfoxide, and 10 mM sodium phosphate buffer, pH 6.8, for 1 h at 50°C. The RNA was applied to 1.2% agarose gel and separated in 10 mM sodium phosphate buffer, pH 6.8, transferred to Hybond N membrane, and immobilized by UV cross-linking. Filters were hybridized in 1% bovine serum albumin, 75% SDS, 0.5 mM sodium phosphate buffer, pH 6.8; 1× EDTA for 14 h at 65°C. The membrane was washed twice at 65°C for 20 min with 0.5% bovine serum albumin, 0.5% SDS, 40 mM sodium phosphate buffer, pH 6.8, 1× EDTA and subsequently twice for 20 min in 1× SDS, 40 mM sodium phosphate buffer, pH 6.8, 1× EDTA. Autoradiography was performed overnight. For hybridization, we used the isolated chicken cDNA fragment (200-bp insert) obtained as described above to verify the RNA loadings used in the analyses, the Northern blots were reproblotted with a labeled 1300-bp cDNA fragment of rat glycerolaldehye-3-phosphate dehydrogenase. Filters were stripped between hybridizations by incubation in 0.1× SSC, 0.1% SDS, 100 mM sodium phosphate buffer, pH 9.5, 65°C. cDNA probes were labeled according to the manufacturer's procedure (Megaprime DNA labeling systems, Amersham Corp.). For quality control, the autoradiograms were scanned using a 1442 laser scanning densitometer (ImageQuant Densitometer, Molecular Dynamics).

Fusion Protein and Antibody Generation—A fusion protein was constructed to generate antibodies against a chicken LCAT peptide. To this end, the LCAT cDNA was fused in-frame to the trpE gene of E. coli (in pPA7 for expression in E. coli in vitro and pP2A for expression in E. coli in vivo). First, a restriction site was introduced into the chicken cDNA clone by site-directed mutagenesis using PCR. The resulting PCR fragment of the expected size of 1690 bp was cut by Smal, and the resulting 1415-bp fragment was blunt-end ligated into the PHTH 10 vector system. DH5α cells were transformed with pATH carrying the LCAT insert (corresponding to bp 348-1763), and the expression of the hybrid fusion protein was induced by tryptophan starvation. Protein extracts from large scale preparations were separated on 8% SDS-polyacrylamide gels. A 69-kDa band (consisting of the antranilate synthase peptide = 37 kDa and the LCAT peptide = 32.3 kDa) was cut out and electroeluted. The eluted protein was concentrated by precipitation in 15% trichloroacetic acid. Approximately 200 µg of the fusion peptide was mixed with complete Freund’s adjuvant and injected into an adult female New Zealand White rabbit. Boosts were performed with 100 µg of protein and incomplete Freund’s adjuvant 2, 4, and 6 weeks after the first injection. Serum was tested by Western blotting. Preimmune serum from the same rabbit was used as control.

Western Blotting—Proteins of plasma fractions enriched in LCAT activity were separated under reducing conditions in 8% SDS-polyacrylamide gels (12) and transferred to nitrocelulose (13). The nitrocelulose membranes were blocked with Blotto (5% dry milk in Tris-buffered saline, 0.1% Triton X-100) and then incubated with rabbit anti-chicken LCAT serum (diluted 1:10,000). The second antibody was goat anti-rabbit IgG conjugated to horseradish peroxidase. Bound antibody was detected with the enhanced chemiluminescence method (14).

Partial Purification of LCAT—Twenty-five ml of laying hen plasma was applied to a phenyl-Sepharose column (bed volume, 20 ml), equilibrated with 0.5 M NaCl. The column was washed with 150 ml of 0.5 M NaCl, and bound proteins were subsequently eluted with water. Aliquots of 1 ml were collected, and 50 µl of each fraction were used for LCAT activity assays. Proteins of the same fractions were separated on an 8% SDS-polyacrylamid gel under reducing conditions and subjected to Western blotting as described above.

RESULTS

Molecular Characterization of Chicken LCAT—Screening of 2 million independent clones of a chicken embryo agt11 cDNA library with a PCR-amplified chicken LCAT cDNA fragment resulted in the isolation of a 1929-bp cDNA insert (Fig. 1A). As shown in Fig. 1B, this clone provided the complete sequence of mature chicken LCAT, albeit not the initiator methionine; the first 66-bp code for the major part of the signal peptide. The amino terminus of the mature enzyme was tentatively assigned by location of the start of the region with almost complete identity to the human enzyme’s sequence (17) (Fig. 1B). The chicken LCAT cDNA sequence encodes a mature 391-residue protein with a calculated molecular mass of 45,422 Da.

The overall similarity at both the nucleotide and amino acid level of the avian enzyme with mammalian LCATs including those of man, baboon, rat, and mouse (17–20) is striking. For instance, the chicken LCAT protein sequence is 73% identical with that of the human enzyme, and amino acids unequivocally involved in LCAT function are conserved. This includes Ser-181 of the catalytically active site (21, 22), contained in the motif GXSGXG. This motif is found not only in LCATs, but also in lipases (lipoprotein lipase, hepatic lipase, lingual lipase, and pancreatic lipase); (23–30) (Table I). In addition, adjacent to the GXSGXG motif the chicken enzyme contains the so-called interfacial binding region (17), an extended linear hydrophobic amino acid stretch (VFLLHGSMDNLNLVFLLL) common to catalytic factors interacting with lipids (29, 31, 32).

Based on these extensive similarities between the avian gene product and its mammalian counterparts, it is surprising and interesting that this conservation does not extend to cysteine residues. Chicken LCAT contains 6 cysteines, whereas all mammalian LCAT harbor 6 cysteine residues. The 4 cysteines known to be involved in intramolecular disulfide-bonds in mammalian enzymes (Cys-50/Cys-74 and Cys 313/Cys-356) are unchanged; the fifth cysteine, in position 26 has been replaced by an ilee in the mammalian enzymes. Significantly and surprisingly, Cys-31 (replaced by Phe) and Cys-184 (substituted by Asn) are absent from the chicken protein. Biochemical data obtained with the human enzyme (33, 34) had suggested that these two cysteines may be crucial for acyl-transfer activity. However, chicken LCAT is catalytically active (see below), strongly supporting earlier site-directed mutagenesis studies (35) that showed that these cysteines are not essential for LCAT activity. In addition, there are no amino acid replacements at sites that when mutated cause classical human familial LCAT deficiencies or fish eye disease (3, 36–45). Also, all of the four potential asparagine-linked glycosylation sites found in mammalian LCATs are conserved in the chicken sequence (Asn-Xaa-Ser/Thr, at residues 20, 84, 272, and 384). The 3′-untranslated region of our clone (690 nucleotides) exceeds that of human LCAT mRNA by 536 nucleotides (17) (Fig. 1A). It contains an additional in-frame stop codon and the polyadenylation signal, AAUAAA. There are also five repeats of...
FIG. 1. A, nucleotide sequence and deduced amino acid sequence of the chicken LCAT cDNA. The amino acid sequence is given in the single-letter code; the residues of the presumed leader peptide are in lower case letters. The in-frame stop codons are underlined once, and the polyadenylation signal (AAUAAA) is underlined in boldface. The tetranucleotide (GAUG), resembling a motif thought to be responsible for the destabilization of specific mRNAs after estrogen withdrawal (see text) is marked with a double underline. B, alignment of the deduced amino acid sequence of chicken and human LCAT. The amino acid sequences are given in the single-letter code and are numbered from the NH₂ terminus of the mature protein (+1). Negative numbers refer to the presumed leader peptide sequence. The shaded areas indicate identical residues in the chicken and human sequences. The 6-residue stretch, identical to the interfacial active binding site of the lipase family, is underlined in boldface. The four potential glycosylation sites are indicated by brackets. The active serine is indicated with an asterisk, and cysteines in the human sequence, not found in the chicken enzyme, are marked with dots.
a tetranucleotide, GAUG, resembling the situation in the 3'-untranslated end of the mRNA for apo-VLDL-II, a strictly estrogen-dependently expressed avian apolipoprotein (46). This tetranucleotide, among other primary and secondary structures, has been suggested to be involved in destabilization of specific mRNAs after estrogen withdrawal (47, 48).

Sites of LCAT Gene Expression—Next, we performed Northern blot analysis of total cellular RNA from different tissues of adult laying hens and mature roosters, respectively (Fig. 2). Hybridization with the 32P-labeled 1929-bp chicken LCAT cDNA fragment revealed a single transcript of approximately 2 kilobase pairs in laying hen liver, brain, and adrenals. Liver and brain of roosters also showed strong signals of the same size, with much lower expression in testes. Control hybridizations were performed with a rat glycerolaldehyde-3-phosphate dehydrogenase probe (Fig. 2, lower panels). This analysis revealed that the tissue distribution of LCAT in the avian female is quite different from that in man, rat, and mouse. As far as has been demonstrated in mammals, LCAT mRNA is predominantly found in liver (17, 20, 49). However, standardization demonstrated that LCAT mRNA levels in the laying hen liver are only 20% of that in brain (Table I). In adult roosters, levels of LCAT mRNA in the liver were higher than in the brain (Fig. 2 and Table I).

Developmental Pattern of LCAT Gene Expression—These data raised the question whether the striking tissue-specific expression of LCAT in female chickens could also be observed in embryonic tissues. Fig. 3 shows the developmental pattern of LCAT mRNA levels in liver and brain. The enzyme is expressed exclusively in liver during embryogenesis (this includes embryos of both sexes), and is by far higher in liver than in brain of female chicks up to 15 weeks after hatching. In the brain, detectable levels of LCAT transcript appear at 11 weeks, increase at 15 weeks, and at maturity (25 weeks; onset of egg laying normally occurs between 20 and 24 weeks), expression in the brain exceeds that in the liver by several fold (cf. also Fig. 2). Thus, the sharp rise in brain LCAT gene expression relative to that in the liver coincides with sexual maturation of the female.

Characterization of LCAT Activity and Protein in Chicken Plasma—LCAT activity in chicken plasma could be detected after partial purification of the enzyme by hydrophobic chromatography. Fig. 4A shows the results of a typical chromatographic separation of laying hen serum proteins on phenyl-Sepharose, generating a 74-fold enrichment of LCAT activity. The fractional esterification rate in the pooled fractions containing the highest activity (fractions 24–32) was 30%, similar to the fractional esterification rate of partially purified human LCAT. Aliquots of each fraction of the phenyl-Sepharose column were analyzed by Western blotting using a rabbit antibody generated against a recombinant chicken LCAT fusion protein as described under “Materials and Methods.” The fractions with the highest enzymatic activity contained an immunoreactive protein with an approximate relative molecular mass of 64 kDa, the amount of which correlated well with LCAT activity (Fig. 4, A and B). The rabbit antibody, obtained early in the immunization scheme, cross-reacted with a second protein (approximately 95 kDa); however, the intensity of this band did not correlate with the level of LCAT activity. It is interesting and useful to note that the antibody generated against the chicken LCAT fusion protein also recognizes human LCAT (Fig. 4C; this anti-serum was obtained following a booster injection and lacks cross-reactivity with the 95-kDa protein). Fig. 4C also documents that chicken and human LCAT comigrate on SDS-gels. Thus, based on the cDNA-derived calculated mass (approximately 45 kDa), the avian enzyme likely is glycosylated to a significant extent. The human enzyme, which has a calculated relative molecular mass (including the carbohydrate moiety) of between 60 and 67 kDa (17) has been reported to have an apparent molecular weight of 68,000 on SDS gels (50, 51).

**DISCUSSION**

Molecular characterization of the first pre-mammalian LCAT has revealed novel details about the structure/function relationships and features of expression of this important enzyme in lipoprotein metabolism. The chicken (G. domesticus), situated in evolution before the emergence of mammals, expresses...
all components identified as essential for the process of reverse cholesterol transport. In fact, in the hen, reverse transport of LCAT-generated cholesteryl esters is not only directed to the liver, but also to the ovary, and is therefore particularly important for normal development of the oocytes, i.e., for reproduction.

The delineation of the primary structure of mature chicken LCAT revealed that it is identical to the most distant known enzyme, that from man, in 284 of its 391 residues (73% identity); at the carboxyl terminus, mammalian enzymes have acquired a proline-rich extension of 23–25 residues. However, all of the residues identified to be important for the catalytic activity of mammalian enzymes are already present in the chicken sequence. LCAT dysfunctions in the human diseases, LCAT deficiency and fish eye disease, have been reported to result from mutations at different sites (3, 36–45, 52, 53). Significantly, all of the residues corresponding to these crucial sites in the human wild-type enzyme are identical to those in chicken LCAT. In addition, all four potential glycosylation sites are conserved in the avian sequence (Asn-Xaa-Thr/Ser, at residues 20, 84, 272, and 384). Mutations introduced by site-directed mutagenesis revealed that substitution of one of these sites (Asn-272) causes diminished LCAT activity (54). All of these findings are in accordance with the consequences of evolutionary pressure to retain functionally important residues.

Special attention has been paid to the role(s) of the cysteine residues in LCAT structure and function. Overall, the chicken enzyme harbors 5 cysteines; one of these (Cys-26) has been replaced by an Ile in the known mammalian enzymes. Through acquisition of two new cysteines (at positions 31 and 184), mammalian LCATs possess 6 cysteines; however, these 2 additional cysteines are present in reduced form (55, 56). The remaining 4 cysteines, common to chicken and mammalian enzymes, are known to form disulfide bonds in the human enzyme (between positions 50 and 74 and positions 313 and 356, respectively (56)), and thus likely in the avian enzyme as well.

Mutations introduced at the paired cysteine sites result in impaired LCAT secretion and activity (56). Early biochemical studies on human LCAT had implied that the free cysteines at position 31 and 184 are also essential for enzymatic activity of
LCAT (33, 34). Chemical modification of the 2 cysteine residues in human LCAT suggested their participation in a complex catalytic mechanism involving an obligatory LCAT-S-acyl intermediate formed in an reaction with both of the respective free cysteine residues (33). These data are not consistent with later findings of Francione and Fielding (35) who generated LCAT which carried mutations in either or both of the free cysteine residues. Even the double mutant (Cys-31 → Gly/Cys-184 → Gly) was active in the formation of cholesteryl esters (35). The ancestral LCAT of chicken, in which these free cysteines are not yet present, is catalytically active. This provides proof for the notion that the cysteine residues in positions 31 and 184 are not required for cholesteryl ester synthesis. It will be interesting to study at which point in evolution, and possibly why, these cysteines arose.

Another significant aspect of the present work relates to the developmental and apparently sex-specific pattern of LCAT gene expression (Figs. 2 and 3). In mature hens, LCAT message is predominantly found in brain, and comparatively low levels are found in liver and adrenals. In contrast, roosters express higher mRNA levels in liver than in brain and very low levels in testes. Thus, in comparison with the rooster and with mammalian systems (20, 57), the laying hen shows a strikingly different and hitherto unrecognized distribution of LCAT transcripts. Lipoprotein metabolism and transport pathways of the hen must ensure sufficient nutritional supplementation of the embryo, i.e. deposition of lipid-rich yolk into growing oocytes. Besides the two major precursors of the yolk components, VLDL and vitellogenin, a bona fide HDL fraction has been demonstrated to constitute a component of egg yolk (6, 58). While VLDL is the predominant lipoprotein fraction of laying hen plasma, the presence of considerable amounts of HDL suggests that the cholesteryl esters derived from plasma HDL also contribute to the yolk cholesterol pool (6). Esterification of cholesterol takes place in the HDL particle by the action of LCAT, underscoring the importance of this enzyme in reproduction.

Upon onset of egg laying, birds show dramatic changes in their lipoprotein profile (59, 60). Immature hens and roosters have low VLDL levels, and in both animals the HDL fraction dominates over the low density fraction. This distribution reverses upon maturation of the female or following estrogen-treatment of roosters; there is an up to 1000-fold induction of certain apolipoproteins and lipid synthetic enzymes (46, 61). We thus wondered whether we could observe, as a corollary to active reverse cholesterol transport, a relationship between the developmental stages of the hen and LCAT expression.

In this context, in embryos, female chicks, and young hens, LCAT gene expression occurs predominantly in the liver, likely correlating with their high plasma HDL levels. Recent reports also demonstrated apoa-I expression in the liver of developing chicks (62). In man, mouse, and rat, the liver is the predominant tissue of LCAT mRNA expression (20, 57). Interestingly, one primate species, the baboon, has been reported to exhibit 3 times higher LCAT mRNA levels in brain than in liver of female animals (18); the authors observed similar results in male baboons. In all other studies so far, extrathoracic tissues were found to express only trace amounts of LCAT (20, 57).

It is probably not unexpected to find LCAT mRNA expression in brain, which is separated from the plasma compartment by the blood-brain barrier and thus from proteins that cannot freely cross this boundary. However, several lines of evidence indicate that the brain maintains cholesterol homeostasis by both uptake as well as removal. Pitas et al. (63) demonstrated that all known components of proper lipoprotein metabolism are present and functional in the brain. ApoE and apoa-I are the major apolipoproteins in human and canine cerebrospinal fluid (63-66). Recently, further apoproteins have been described from human cerebrospinal fluid, such as apoa-IV, D, and (67). Since the adult brain does not accumulate cholesterol, it must possess a mechanism(s) to dispose of excess cholesterol. ApoA-I-carrying HDL particles in conjunction with LCAT may well participate in this reverse cholesterol transport process. Notably, LCAT, albeit with low activity, has been demonstrated in human cerebrospinal fluid (68). In brain of laying hens, we found dramatically increased LCAT mRNA levels compared with liver, possibly reflecting the need for maintenance of cholesterol homeostasis in this partially closed system. LCAT action in brain may be important for function of the central nervous system, since several patients with LCAT deficiency showed signs of hearing loss and sensory impairments (4). Whether higher levels of LCAT mRNA expression correlate with high enzyme activity in the brain of adult chickens and the baboon (18) needs to be addressed in further studies.

The observed tissue-specific expression of LCAT may be subject to tight control. For instance, in addition to known promoter elements (69), we might find tissue-specific posttranscriptional control of the LCAT gene. We have observed in the 3′-untranslated region of chicken LCAT elements previously characterized as important for estrogen regulation at the level of mRNA stability (47, 48). Thus, the chicken with its dramatic developmental changes and gender differences in LCAT gene expression provides a powerful system to delineate possibly novel control elements of LCAT regulation in vivo.

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