review

# Role of apoA-II in lipid metabolism and atherosclerosis: advances in the study of an enigmatic protein

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**Abstract** **Our understanding of apolipoprotein A-II (apoA-II) physiology is much more limited than that of apoA-I. However, important and rather surprising advances have been produced, mainly through analysis of genetically modified mice. These results reveal a positive association of apoA-II with FFA and VLDL triglyceride plasma concentrations; however, whether this is due to increased VLDL synthesis or to decreased VLDL catabolism remains a matter of controversy. As apoA-II-deficient mice present a phenotype of insulin hypersensitivity, a function of apoA-II in regulating FFA metabolism seems likely. Studies of human beings have shown the apoA-II locus to be a determinant of FFA plasma levels, and several genome-wide searches of different populations with type 2 diabetes have found linkage to an apoA-II intragenic marker, making apoA-II an attractive candidate gene for this disease. The increased concentration of apoB-containing lipoproteins present in apoA-II transgenic mice explains, in part, why these animals present increased atherosclerosis susceptibility. In addition, apoA-II transgenic mice also present impairment of two major HDL antiatherogenic functions: reverse cholesterol transport and protection of LDL oxidative modification. The apoA-II locus has also been suggested as an important genetic determinant of HDL cholesterol concentration, even though there is a major species-specific difference between the effects of mouse and human apoA-II. As antagonizing apoA-I antiatherogenic actions can hardly be considered the apoA-II function in HDL, this remains a topic for future investigations. We suggest that the existence of apoA-II or apoA-I in HDL could be an important signal for specific interaction with HDL receptors such as cubilin or heat shock protein 60.**—Blanco-Vaca, F., J. C. Escolà-Gil, J. M. Martín-Campos, and J. Julve. **Role of apoA-II in lipid metabolism and atherosclerosis: advances in the study of an enigmatic protein.** ***J. Lipid Res.*** **2001.** 42: **1727–1739.**

**Supplementary key words** apoA-I • apoB • HDL • hyperlipidemia • hypertriglyceridemia • insulin resistance • triglycerides • type 2 diabetes • VLDL

## THE GENE

### Structural organization

Apolipoprotein A-II (apoA-II) is a member of the apolipoprotein multigene superfamily, which includes genes encoding soluble apolipoproteins (apoA-I, apoA-II, apoC's, and apoE). The human *apoA-II* gene has been cloned and sequenced in several organisms and has been mapped in humans to chromosome 1 in the region 1p21→1qter (1). Interestingly, in the mouse, the *apoA-II* gene was also shown to reside on mouse chromosome 1 in a fairly large region of synteny (2). ApoA-II shares with other soluble apolipoproteins its genomic structure of four exons and three introns (**Fig. 1**), the latter located at similar positions. The first intron is situated in the 5′ untranslated region of the gene, 24 bp upstream of the initiator methionine codon. The second intron is positioned close to the signal peptidase cleavage site, whereas the third intron, the largest, divides the region encoding the mature protein into two portions, possibly with different functions. Exon 4 encodes the C-terminal domain of the protein, containing several α-helical repeats of 22 amino acids. These similarities in the structural organization of soluble apolipoproteins suggest that they have evolved from a common ancestor by duplication/deletion of a 22-amino acid repeat (1).

Some polymorphisms in the *apoA-II* gene region have been identified (Fig. 1). Close to the acceptor splice site of intron 2 is located a polymorphic GT repeat (3), which does not appear to have an effect on the splicing process (4). This intragenic polymorphism is used as a marker in linkage studies. Also, three single-nucleotide polymorphisms have been described in the *apoA-II* gene region: a

Abbreviations: apo, apolipoprotein; ABC-1, ATP-binding cassette transporter 1; ARP-1, apolipoprotein regulatory protein 1; CAD, coronary artery disease; C/EBP, CCAAT enhancer-binding protein; CERP, cholesterol efflux regulatory protein; CLA-1, CD36 and LIMPII analogous 1; EAR, v-Erb-related receptor; HL, hepatic lipase; HFN-4, hepatic nuclear factor 4; Hsp60, heat shock protein 60; LpA-I, HDL containing apoA-I but not apoA-II; LpA-I/A-II, HDL containing apoA-I and apoA-II; LpA-II, HDL containing apoA-II but not apoA-I; PLTP, phospholipid transfer protein; PPAR, peroxisome proliferator-activated receptor; PAF-AH, platelet-activating factor acetylhydrolase; RXR, retinoid X receptor; SR-BI, scavenger receptor class B type I; SREBP, sterol regulatory element binding protein; USF, upstream stimulatory factor.

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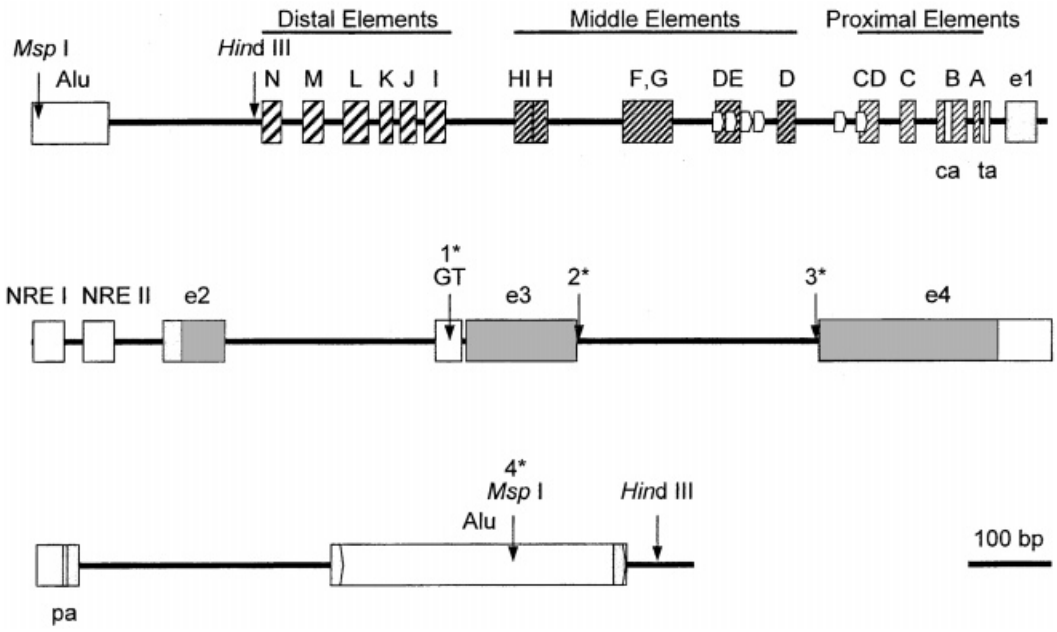


**Fig. 1.** Schematic representation of the *apoA-II* gene. Several regulatory elements are located in the promoter region (−911 to +29), which can be divided into three functional regions (see text for details). Arrows indicate the presence of direct repeats in the promoter region. Also, the first intron appears to contain negative regulatory elements (NRE I and II). ca, CAAT box; ta, TATA box; pa, polyadenylation signal. Polymorphic sites are indicated by an asterisk: 1*, GT repeat region; 2*, G-to-A transition that affects the donor splice site of third intron; 3*, C-to-T transition near the acceptor splice site of intron 3 that also modifies a *Bst*NI restriction site; 4*, C-to-T transition that modifies an *Msp*I restriction site within the 3′ Alu element.

G-to-A transition affecting the donor splice site of intron 3, named apoA-II$_{Hiroshima}$ and associated in homozygosis with familial apoA-II deficiency (5), a C-to-T transition near the acceptor splice site of intron 3, modifying a *Bst*NI restriction site (6), and a C-to-T transition within the 3′ *Alu* element, modifying an *Msp*I restriction site (7). The possible importance of these polymorphisms is discussed below.

### Transcriptional regulation

In humans, mice, and rats, apoA-II is synthesized mainly by the liver and, to a much lesser extent, by the intestine (8). Several studies have shown that the transcription of the human *apoA-II* gene is controlled by a complex array of proximal, middle, and distal regulatory elements, designated from A to N, situated in the *apoA-II* promoter region (Fig. 1). These elements, located between nucleotides −903 and −33, can be separated into eight proximal and middle (AIIA to AIIH) and six distal (AIII to AIIN) elements (9). This region is sufficient to restrict *apoA-II* gene expression to the liver in transgenic mice (10). The distal elements, between nucleotides −903 and −680, act as enhancers when placed in front of homologous and heterologous liver-specific promoters (11, 12). Thus, liver-specific expression of the *apoA-II* gene is controlled by the synergistic interactions of factors that bind to the proximal and distal regulatory elements (13). Also, regulatory elements exist outside the promoter region. Transfection of HepG2 and CaCo-2 cells has shown that the first intron of the *apoA-II* gene acts as a silencer, reducing the transcription driven by of the *apoA-II* promoter to 15–18% of its original value (14).

An important factor for the activity of the *apoA-II* promoter is the ubiquitous heat-stable upstream stimulatory factor (USF), previously designated CIIIBI, which binds to the regulatory elements AIIB, AIIK, and AIIL (9). Cotransfection studies performed in HepG2 cells have established that the USF1/USF2a heterodimer, which is expressed in the liver, is as efficient as the USF2a homodimer in the *trans*-activation of *apoA-II* promoter-enhancer constructs (15). Elements AIIA and AIIK bind, in addition to the USF, a heat-labile activity designated AIIABI (13). Also, the CCAAT enhancer-binding protein (C/EBP), a heat-stable protein that was initially identified in rat liver nuclear extracts, binds with high affinity to regulatory elements AIIL, AIIC, and AIID and with less affinity to IIF, AIIG, and AIIA (16).

An important role in *apoA-II* gene regulation is also exerted by the hormone response element present in the regulatory element AIIJ, which contains two imperfect copies of a motif related to the consensus steroid hormone receptor half-site TGACCT with one-nucleotide spacing (17, 18). Several studies have shown that orphan nuclear receptors, such as hepatic nuclear factor 4 (HNF-4), v-Erb-related receptor 2 (EAR-2), EAR-3, and apolipoprotein regulatory element 1 (ARP-1), also interact with this J site (16, 17). HNF-4 induces, whereas EAR-2, EAR-3, and ARP-1 reduce, *apoA-II* gene transcription on binding to this element (17). Interestingly, cotransfection experiments in COS-1 cells showed that HNF-4 synergized with USF2a in the *trans*-activation of the *apoA-II* promoter and that they bind to the enhancer cooperatively (15). The *apoA-II* promoter is also *trans*-activated by retinoid X receptor (RXR) α ligands

(9-*cis*-retinoic acid and its precursor all-*trans*-retinoic acid) and agonists (i.e., LGD 1069) (18), and also, by fatty acid derivates and fibrates that act on the element AIIJ in vivo via activation of the peroxisome proliferator-activated receptor α (PPARα) (19), strongly suggesting that the heterodimer RXR-PPARα could be a positive physiological regulator of the *apoA-II* transcriptional rate.

Several studies have shown that sterol regulatory element binding protein 1 (SREBP-1) and SREBP-2 recognize regulatory elements AIIB, AIICD, AIIDE, and AIIK and that SREBP-1 also binds to AIIHI (20, 21). These elements contain inverted palindromic or direct repeat motifs and bind SREBP with different affinities. However, their role in the in vivo transcription of the *apoA-II* gene has not been established.

From these data, it can be deduced that plasma levels of apoA-II can be altered by altering its gene transcription levels in response to intra- and extracellular stimuli (metabolites and hormones) and this may be physiologically important as apoA-II plasma levels are more influenced, at the population level, by its synthesis rate rather than by its catabolism (22). A good example of this is the report of low apoA-II, apoC-III, lipoprotein [a], and triglyceride levels of individuals heterozygous for an HNF-4α mutation (23). From a pharmacological point of view, fibrates have been shown to increase human apoA-I and apoA-II synthesis through a PPAR-RXR-mediated mechanism, whereas the same mechanism caused a decrease in these HDL apolipoproteins in mice (18).

Further details about the contribution of all these factors to the transcriptional regulation of apoA-II synthesis can be obtained from an excellent review (9). Much less is known about posttranscriptional regulation of apoA-II (24).

## THE PROTEIN

### Structural characteristics, synthesis, and secretion

The human apoA-II protein sequence is known (25). The major molecular species of human apoA-II in plasma is the homodimer formed by two polypeptide chains of 77 amino acids linked by a disulfide bond at residue 6 (25). Other molecular species containing apoA-II also exist in plasma. These are the apoA-II-apoE (which exists in individuals with at least one allele, *apoE2* or *apoE3*, encoding one and two cysteines, respectively) (26, 27) and apoA-II-apoD heterodimers, and the apoA-II monomer (28). The theoretical basis of disulfide bond formation among cysteine-containing apolipoproteins has been discussed elsewhere (28).

Early studies showed that association of phospholipids with apoA-I and apoA-II results in an increase in the α-helical content of the protein as measured by circular dichroism (1). Computer analysis of the different classes of amphipathic helices has led to classification of the physicochemical properties associated with each class (29). Class A is characterized by a high mean hydrophobic moment and by its unique charge distribution. Positively charged residues are clustered at the polar-nonpolar interface, whereas negatively charged residues are found at the center of the polar face (29). Three such helices with this specific amino acid distribution (helices 7–30, 39–50, and 51–70) had been identified in apoA-II (29). Experiments with synthetic peptides have suggested that there are at least two lipid-associating domains in apoA-II located at opposite ends of the molecule, between amino acids 12–31 and 40–77 (29). All these studies, and the fact that soluble apolipoproteins act as protein detergents, support the idea that these amphipathic helices serve in general as lipid-associating protein detergent domains.

The primary translation product of apoA-II mRNA consists of 100 amino acids containing an 18-residue-long signal peptide (1). The newly secreted apoA-II consists of 82 amino acid residues and is converted to the mature form by proteolytic cleavage of a 5-residue-long prosegment (Ala-Leu-Val-Arg-Arg) attached to the $NH_2$ terminus (1). This prosegment is cleaved intra- and extracellularly by a thiol protease to produce the mature protein form (1). The prosegment may serve as a signal for apolipoprotein targeting along the secretory pathway, extracellular processing, or maturation of nascent lipoproteins (8). ApoA-II has no consensus sequence for N-linked glycosylation and in plasma is not significantly glycosylated. However, in lymph and in HepG2 culture medium about half of the apoA-II is O-glycosylated. O-linked glycosylation has been shown not to be necessary for secretion, but reduces association with HDL of the newly synthesized apoA-II (30).

### Plasma concentration, association with lipoproteins, and metabolism

ApoA-II is the second major protein constituent of HDL, accounting for about 20% of HDL protein. The mean apoA-II plasma concentration in normolipidemic humans is about 30–35 mg/dl (31), although more than 20% of patients with coronary artery disease (CAD) seem to have concentrations between 40 and 60 mg/dl (18, 31).

ApoA-II is mainly found associated with HDL, with a (usually small) fraction associated with chylomicrons and VLDL (32). The major HDL subclasses are those containing apoA-I but not apoA-II (LpA-I) and those containing both apoA-I and apoA-II (LpA-I/A-II) and can be determined directly with an enzyme-linked differential antibody immunosorbent assay (33). A minor part of apoA-II is in HDL particles that do not contain apoA-I (LpA-II) (34). The relative proportion of LpA-I is greater in $HDL_2$ and that of LpA-I/A-II is greater in $HDL_3$ (33). The concentration of LpA-I/A-II appears to be related to the synthesis of apoA-II in normolipidemic humans (22). In support of that, there exists a mouse polymorphism of apoA-II that affects apoA-II translational efficiency and, indeed, increases the apoA-II/apoA-I ratio (35). Formation of LpA-I/A-II seems to take place in plasma and could require an LCAT-dependent LpA-I/LpA-II particle fusion process (36). Other possible mechanisms for the formation of LpA-I/A-II include the displacement of apoA-I by free apoA-II (22, 29, 36). There is no evidence that free apoA-II is formed during LpA-I/A-II metabolism, and whether catabolism affects the whole LpA-I/A-II particle

or its parts is unknown (36). Furthermore, in contrast to apoA-I, no renal excretion of apoA-II has been demonstrated (36).

### Comparison among different animal species

ApoA-II is present in the plasma of humans, simians, mice, rats, and fish. However, for unknown reasons, apoA-II is either absent or expressed at a low level in dogs, pigs, chicken, and rabbits (1, 37).

The two animal species in which apoA-II has been studied most are humans and mice. There are marked differences between human and murine apoA-II. Human apoA-II exists as a disulfide-linked homodimer, whereas murine apoA-II (and apoA-II of other known animal species) exists exclusively as a monomer because of the absence of a cysteine residue at position 6 (38). Furthermore, human and murine apoA-II amino acid sequences differ by approximately 40%, which explains why monomeric human apoA-II expression in transgenic mice does not have effects similar to those of murine apoA-II (38, 39).

## ROLES IN LIPID AND LIPOPROTEIN METABOLISM

### Roles in HDL structure-function and metabolism

*Role in determining HDL particle size and HDL cholesterol concentration.* HDL are subjected to continual remodeling in plasma and there is evidence suggesting that apoA-II may play an important role in this process. As analyzed in detail below, HDL remodeling may be determined in part by the influence of apoA-II on the reactivity toward lipid transfer proteins, enzymes, and the receptors involved in HDL metabolism and, also, by its ability to displace apoA-I from the HDL surface even though this changes HDL reactivity toward one of the above-mentioned enzymes, LCAT. The latter effect has been described in vitro in both isolated and reconstituted HDL (40–42). Studying the effect of overexpression, or knocking out, of apoA-II in genetically modified mice has provided insight into the species-specific role of this apolipoprotein in HDL remodeling. Overexpression of human apoA-II in transgenic mice induced the appearance of smaller HDL particles that were heterogeneous in size, similar to those present in human plasma and in contrast to the monodisperse HDL population typical of the mouse (43, 44). Interestingly, human apoA-I expression in transgenic mice also induced HDL size heterogeneity (45), suggesting that HDL particle size species-specific differences depend at least on apoA-I and apoA-II structure. In support of this interpretation, murine apoA-II expression in transgenic mice induced the presence of HDL particles larger than those of control mice (46), and apoA-II-deficient mice presented HDL of decreased particle size (47). In all these studies, the presence of larger HDL particles in plasma was concomitant with increased concentrations of HDL cholesterol, whereas smaller HDL particles were associated with decreased HDL cholesterol (**Table 1**) (43, 44, 46, 47). However, the only human family known to have apoA-II deficiency exhibited normal levels of plasma lipids and lipoproteins (5). Despite the marked differences in the effects of human and murine apoA-II on plasma HDL (5,43, 44, 46, 47), the different behavior of mouse and human apoA-II provides us with a unique species-specific perspective of apolipoprotein function. It is likely that the different effects of mouse and human apoA-II depend, at least in part, on different interaction with other proteins involved in lipoprotein metabolism such as enzymes, lipid transfer proteins, and receptors.

*Role in cholesterol efflux.* HDL apolipoproteins located in the extracellular space are potentially important players in cholesterol efflux from peripheral cells, which is the first step of reverse cholesterol transport (48). Cholesterol efflux can occur either by a passive slow diffusion-type mechanism or by a fast specific energy-dependent mechanism that results in the transfer of cholesterol to lipid-poor apolipoproteins (49). Past controversies about the role of LpA-I and LpA-I/A-II could have been due, at least in part, to use of different cell types, different acceptors (free apolipoproteins, isolated lipoproteins, or whole serum), and different incubation times. There is now some consensus that LpA-I is more active than LpA-I/A-II in reverse cholesterol transport. This is likely to be due to its ability to induce both fast specific and slow nonspecific cholesterol efflux, whereas LpA-I/LpA-II would induce mainly the latter (49). Also, and perhaps in relation to this reduced ability to induce cholesterol efflux, apoA-II has been specifically found to induce decreased phospholipid efflux compared with that induced by apoA-I (50). Several studies of genetically modified mice that express or do not express human apoA-I and apoA-II have provided additional insights into this topic. Expression of murine apoA-II in transgenic mice did not increase cholesterol efflux from macrophages compared with controls, which is in contrast to the results obtained in human apoA-I transgenic mice (51). In one study by our group, cholesterol efflux induced by serum of mice overexpressing human apoA-II from NRK fibroblasts was decreased compared with respect to control mice when they were fed a regular chow diet but not when they were fed a high fat, high cholesterol-containing diet (52). The former diet was concomitant with the presence of smaller HDL particles and greater LCAT and HDL deficiency (in both cholesterol and phospholipid content) of the transgenic mice (52). However, other studies of our transgenic mice (J. Julve, J. C. Escolà-Gil, C. Mayoral, and F. Blanco-Vaca, unpublished results) and of independently generated apoA-II transgenic mice found a decrease in efflux in both types of diets using the same assay and Fu5AH hepatoma cells (53). In addition, another study showed that plasma from human apoA-I transgenic mice was more effective in inducing efflux from the Fu5AH hepatoma cells and Ob1771 adipose cells than plasma from human apoA-I/apoA-II transgenic mice (54). Nevertheless, probably the single most convincing study addressing this point was performed with apoA-I-deficient mice in which efflux, measured also in Fu5AH cells, was decreased compared with control mice (55). Expression of human apoA-I, but not expression of apoA-II, improved cholesterol efflux in apoA-I-deficient mice (55).

TABLE 1. Results of expressing apoA-II in transgenic mice: lipid metabolism and atherosclerosis susceptibility

| Expression | Transgene[a] | Crossed With/Expressed In | Diet | ApoA-II Levels | HDL-C | TG | Non-HDL-C | Atherosclerosis | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *mg/dl* | | | | | |
| Human apoA-II | A and B | ∅ | Chow[b] | 20–50 | N | N | N | ND | 43 |
| | B | ∅ | Chow (fed animals) | 68 | ↓ | ↑ | N | ND | 60 |
| | B | Human CETP transgenic | Chow (fed animals) | 71 | ↓ | N | ↑ | ND | 60 |
| | B | Human apoA-I transgenic | Atherogenic | 108 | N | ND | N | ↑ | 109 |
| Murine apoA-II | C | ∅ | Chow | 60–84 | ↑ | ↑ | ↑ | ↑ | 46, 98 |
| | C | ∅ | Atherogenic | 48–52 | ↑ | ↑ | ↑ | N or ↑ | 98 |
| Human apoA-II | D | ∅ | Chow | 70–85 | ↓ | ↑ | ↑ | N | 44, 52 |
| | D | ∅ | Atherogenic | 91–111 | ↓ | ↑ | ↑ | ↑ | 71, 100 |
| | D | ApoE-deficient mice | Chow | 66–78 | ↓ | ↑ | ↑ | ↑ | 96 |
| | D | Simian CETP transgenic | Chow | 42 | ↓ | ↑ | ↑ | N or ↑ | 61 |
| | D | Simian CETP transgenic | Atherogenic | 107 | ↓ | ↑ | ↑ | N or ↑ | 61 |
| Human apoA-II | E | ∅ | Chow | 43 | N | ↑ | ↓ | ND | 53 |
| | E | ∅ | Atherogenic | 36 | ↓ | ↑ | ↓ | ↓ | 53 |
| Human apoA-II | A[c] | ∅ | Chow (fed animals) | 35–105 | ↓ | ↑ | ↑ | ND | 97 |
| ApoA-II deficiency | F | ∅ | Chow | 0 | ↓ | N | ↓ | ND | 47 |

Abbreviations: HDL-C, HDL cholesterol; N, normal; ND, not determined; ∅, control mice.

[a] The *apoA-II* gene constructions: A, a 3-kb genomic *Hin*dIII fragment containing the human *apoA-II* gene (see Fig. 1 for details); B, a 5-kb construct containing the 5′ upstream promoter and regulatory region of the human *apoA-I* gene (includes exon I and the first 54 bp of intron I) fused to 2 kb of the human *apoA-II* structural gene (includes sequences beginning 5′ of the *Stu*I site within the first intron and extending to the *Hin*dIII site that lies 3′ of the *apoA-II* structural gene); C, a 15-kb mouse *apoA-II* genomic fragment contains 4.5 kb of sequence 5′ of the first exon of the *apoA-II* gene and 8.5 kb 3′ of the last exon of the gene; D, a 3-kb genomic *Msp*I fragment containing the human *apoA-II* gene (see Fig. 1 for details); E, a 700-bp fragment of the human antithrombin III promoter juxtaposed to a human *apoA-II* gene coding region (includes sequences beginning 5′ of the *Bgl*I site, close to the beginning of the second exon of the *apoA-II* gene and extending to the *Hin*dIII site that lies 3′ of the *apoA-II* gene); F, the neomycin resistance gene cassette replaces a 12-kb *Eco*RI fragment of the endogenous locus containing the entire mouse *apoA-II* gene, including the promoter region.

[b] If not otherwise indicated under Diet, mice were studied after they had been fasted.

[c] Independently (from previous A) generated 3-kb genomic *Hin*dIII fragment containing the human *apoA-II* gene.

To date, the main HDL receptor known to be related to cholesterol efflux is ATP-binding cassette transporter 1 or cholesterol efflux regulatory protein (ABC-1/CERP), whereas scavenger receptor class B type I or CD36 and LIMPII analogous 1 (SR-BI/CLA-1) may also be of importance in this process (48). Apolipoprotein acceptor specificity for lipid efflux induced by ABC-1 has been examined in stably transfected HeLa cells expressing human ABC-1 product (56). Like apoA-I and other soluble apolipoproteins, apoA-II was shown to display greater cholesterol and phospholipid efflux capacity from ABC-1-transfected cells than with control cells (56). This increase in efflux seems to correspond to increased binding of both apolipoproteins to a common binding site (56). In contrast, we know of no data regarding the role of apoA-II in SR-BI-mediated cholesterol efflux.

*Role in modulating HDL interaction with lipid transfer proteins and enzymes.* CETP. The effect of the apoA-II moiety of HDL on CETP activity has been studied in vitro by measuring the rate of radiolabeled cholesteryl ester transfer between LDL and $HDL_3$ containing various proportions of apoA-I and apoA-II (57). In this study, the rates of cholesteryl ester exchange induced by purified human CETP from apoA-II-enriched $HDL_3$ to LDL and from LDL to apoA-II-enriched $HDL_3$ were both significantly reduced (57). As observed with $HDL_3$ artificially enriched with apoA-II, cholesteryl ester transfer rates to LpA-I/A-II were significantly decreased with respect to those of LpA-I (57). However, no effect depending on apoA-I or apoA-II content was seen when using reconstituted HDL (58). When studying transgenic mice expressing human apoA-I and/or apoA-II, heat-labile lipid transfer inhibitory activity was found in human apoA-II transgenic mice but not in mice expressing human apoA-I or both human apoA-I/apoA-II (59). However, this study used isolated HDL and inhibition of CETP activity was not evident in vivo in two other studies of apoA-II/CETP transgenic mice (60, 61). In both studies, an important redistribution of cholesterol from HDL to VLDL and triglyceride from VLDL to HDL was seen even though the accumulation of triglyceride in HDL could have been influenced by hepatic lipase (HL) inhibition (60).

PHOSPHOLIPID TRANSFER PROTEIN (PLTP). PLTP binds both purified apoA-I and apoA-II (62). The PTLP effect on HDL size conversion was seen both in LpA-I and in LpA-I/A-II (63). However, an in vitro study showed that a decreased apoA-I/A-II molar ratio in the HDL particle inhibited PLTP-mediated HDL conversion, but not phospholipid transfer reactivity, suggesting that apoA-I is necessary for particle fusion (64).

LCAT. LCAT reactivity is higher in HDL reconstituted with apoA-I than in those reconstituted with apoA-II (42). This is due to the ability of apoA-II or its C-terminal end to displace apoA-I (at high apoA-II/apoA-I ratios) or to destabilize apoA-I (at the physiological apoA-II/apoA-I ratio of 0.5:1), which, in the latter case, becomes more easily exchangeable although this would not result in HDL particle rearrangement (41, 42, 65). This has been attributed, depending on the study, to a decrease in $K_m$ or to a decrease in $V_{max}$ (41, 42, 65). This could indicate that LCAT binding is sterically hindered by portions of apoA-I and apoA-II and would not bind to HDL lipids (41, 42, 65). Therefore, apoA-II could modulate the reaction of HDL with LCAT by decreasing binding to LpA-I/A-II and making the enzyme available for reaction with other lipoprotein particles (65). This interpretation is consistent with the fact that a larger proportion of LCAT is bound to LpA-I rather than to LpA-I/A-II in human plasma (66) and with a report showing, in LCAT transgenic mice with concomitant expression of human apoA-I, human apoA-II, or both, a significant preference of human LCAT for LpA-I particles (67). In addition, another study found a partial apoA-I deficiency with a concomitant functional LCAT deficiency in transgenic mice overexpressing human apoA-II probably because LCAT, although was present in the plasma of the mice, was largely not functional (44, 52). This induced a mild but significant accumulation of free cholesterol in the cornea of these animals (52).

HL. ApoA-II has been suggested to inhibit, to activate, or to have no effect on HL-catalyzed hydrolysis of phospholipids and triglycerides when using artificial lipid emulsions, reconstituted HDL, or HDL isolated from plasma (68). At least part of the contradictory results obtained by these studies may have been due to the different lipoprotein complexes used and to the different measurements performed (hydrolysis of triglycerides, phospholipids, or both). More detailed studies have shown that HL has much more affinity (considering hydrolysis of either phospholipids or triglycerides) for LpA-II whereas, in general, $V_{max}$ was higher for LpA-I (69). However, apoA-I (irrespective of whether it was present in the same particle—as LpA-I/A-II—or was added as LpA-I to the incubation of HL and LpA-II) increased the hydrolysis of phospholipid from LpA-II (70). To shed further light on the in vivo role of apoA-II in HL activity, studies using apoA-II transgenic and knockout mice have been reported. Whereas studies of human apoA-II transgenic mice reached contradictory conclusions, reporting either HL activation (71) or inhibition (60), others using mouse apoA-II transgenic (72) and apoA-II-deficient mice crossed with HL-knockout mice provided convincing evidence that mouse apoA-II inhibits HL (47, 73). However, apoA-II deficiency causes HDL deficiency in mice (47) but not in humans (5), suggesting that the effect of apoA-II on HL could be different in humans and mice. In fact, one study performed with plasma HDL isolated by immunoaffinity chromatography showed higher reactivity of HL toward LpA-I/A-II than toward LpA-I (74) and expression of murine and human apoA-II transgenic mice—in the latter, independent of whether or not they caused LCAT deficiency—originated opposite effects in HDL particle size (43, 44, 46, 47). One of the differences between the HL of both species is that an important proportion of HL is bound to HDL in mouse plasma whereas in humans HL is bound to the liver.

*Role in modulating HDL interactions with receptors.* The role in modulating HDL interactions with receptors has been reviewed (75). HDL interaction with receptors presents characteristics that render their study difficult. One is that

HDL receptors usually exhibit broad specificity for soluble apolipoproteins, a phenomenon that may be explained by the existence of common domains. A second is that the relationship between HDL and cell receptors does not involve "classic" receptor interaction (75).

SR-BI/CLA-1. SR-BI-mediated HDL cholesteryl ester-selective uptake appears to require direct binding of the HDL particle by SR-BI. The influence of apoA-II on selective cholesteryl ester uptake has been addressed (76–78). It has been reported that LpA-I/A-II was associated to a lesser extent than LpA-I with HepG2 cells and fibroblasts and promoted less selective lipid uptake than LpA-I (76). In vitro apoA-II enrichment of HDL was found to actually increase HDL cell association but, in an inverse manner, to decrease selective uptake of cholesteryl ester in an adrenal cell line (77). These results contrast with another study that found reduced association but increased lipid uptake of reconstituted HDL containing apoA-II in SR-BI-transfected Chinese hamster ovary (CHO) cells (78). Comparisons among studies are difficult given the differences in HDL and cell systems used in the three studies. However, in contrast to the studies of CHO cells (clone ldlA7) stably transfected with human SR-BI (CHO-SRBI), studies of HepG2 (76) and adrenal (77) cells did not determine SR-BI-specific events and other HDL receptors could have contributed to the results obtained. The same reasoning can be applied to our observations that autologous HDL labeled with cholesteryl oleyl ether, isolated from and reinjected into mice overexpressing apoA-II or into control mice, are catabolized faster in the former because of increased liver clearance that is concomitant with increased excretion of bile lipids ( J. Julve, J. C. Escolà-Gil, F. González-Sastre, and F. Blanco-Vaca, unpublished results). Experiments are being conducted in our laboratory to ascertain whether increased SR-BI-mediated lipid uptake is the cause of these findings.

CUBILIN. Cultured yolk sac cells incubated with purified apoA-II have reduced HDL uptake (79), but purified apoA-II shows no significant binding to cubilin (79). The inhibitory effect of apoA-II on HDL uptake by cubilin might, therefore, have been due to displacement of apoA-I from the HDL particle instead of direct competition with apoA-I for binding to cubilin (79).

HEAT SHOCK PROTEIN 60 (HSP60). Hsp60 has been shown to be a high affinity HDL-binding protein, especially through binding of apoA-II (80). This is of interest because it is another receptor showing a clearly different preference either for apoA-I or apoA-II. Hsp60 is a highly conserved protein that is most abundant in mitochondria, although it is also expressed in the plasma membrane of at least some of the numerous tissues in which it is expressed (80). Hsp60 forms an oligomeric cagelike structure with two rings of eight subunits, with each ring surrounding a central cavity that is too small to accommodate mature HDL particles. Another aspect of interest in relation to this topic is that autoimmunity toward Hsp60 induces atherosclerosis (80), as is commented on later in this review in the case of apoA-II transgenic mice.

*Role in HDL protection against LDL oxidative modification.* HDL protection of LDL oxidative modification is thought to involve the HDL-associated enzymes paraoxonase and platelet-activating factor acetylhydrolase (PAF-AH), which may act coordinately, and LCAT. Paraoxonase and LCAT are contained mainly in LpA-I particles, whereas PAF-AH is principally in LDL and, in a minor proportion, in very high density lipoproteins (81). This enzyme distribution does not indicate critical participation of LpA-I/A-II particles in the prevention of LDL oxidation. In fact, HDL from murine apoA-II transgenic mice is not only unable to protect against LDL oxidation, but is also a proinflammatory particle, only in part owing to a decrease in paraoxonase content (51, 72). Our data on human apoA-II transgenic mice with LCAT functional deficiency indicate decreased HDL protection against LDL oxidative modification, even though isolated HDL would not become more oxidized than control HDL (V. Ribas, J. L. Sánchez-Quesada, J. Ordoñez, and F. Blanco-Vaca, unpublished data).

*Role in triglyceride and FFA metabolism.* Some population studies of the apoA-II *Msp*I polymorphism, located within an *Alu* element 538 bp downstream the coding region, have shown significant associations with plasma triglycerides (**Table 2**) (82–84), whereas other population studies failed to find this association (6, 85–89). Because the mutation responsible for the *Msp*I polymorphism does not alter the protein sequence, the possible effect of the mutation would be produced at the expression level. Only two studies have found a significant association between *Msp*I polymorphism and apoA-II plasma level (7, 83). Curiously, although the effect of the rare allele ($M^-$) on the level of plasma apoA-II concentration is different in both studies, the apoA-I/apoA-II ratio was found to be significantly decreased in the homozygous $M^-/M^-$ state in both cases, a reduction that was observed in another study (86). Thus, a possible explanation for the relation between *Msp*I polymorphism and plasma triglyceride concentration is the effect that a decrease in the apoA-I/apoA-II ratio could have on the triglyceride-rich particles, but this point is controversial. What does not seem so controversial is the possibility that the *Msp*I rare allele is in linkage disequilibrium with a functional mutation that influences the translational efficiency of apoA-II mRNA (7, 89). Indeed, the *apoA-II* locus has been linked to a locus controlling plasma levels of apoA-II and FFA in both mice and humans (90). However, the *Msp*I polymorphism determines only 7.2% of the variation of plasma apoA-II in the population and this may be too low to obtain consistent results in different studies (91). Interestingly, an early report showed a positive correlation ($r = 0.71$, $n = 14$) between the synthesis of apoA-II and VLDL-apoB in human beings, whereas the latter parameter did not correlate with plasma apoA-II concentrations (92). A locus in the vicinity of the human *apoA-II* gene has been linked to type 2 diabetes in genome-wide searches of French whites, Utah whites of northern European ancestry, and Pima Indians (93–95).

A direct effect of apoA-II on triglyceride and FFA metabolism is, in general, supported by the studies performed in transgenic mice. Mice expressing human apoA-II present a clear increase in cholesterol and/or triglycerides

TABLE 2. Allele frequencies of *Msp*I polymorphism in the *apoA-II* gene as determined in various studies

| | n | $M^+/M^+$ | $M^+/M^-$ | $M^-/M^-$ | $M^+$ | $M^-$ | Reference |
|---|---|---|---|---|---|---|---|
| Control population | | | | | | | |
| British | 87 | 0.71 | 0.20 | 0.09 | 0.81 | 0.19 | 7 |
| British | 59 | 0.66 | 0.31 | 0.03 | 0.81 | 0.19 | 82 |
| British | 101 | 0.67 | 0.31 | 0.02 | 0.83 | 0.17 | 113 |
| British | 85 | 0.69 | 0.28 | 0.02 | 0.84 | 0.16 | 85 |
| Norwegian | 170 | 0.72 | 0.26 | 0.02 | 0.85 | 0.15 | 88 |
| British | 70 | 0.74 | 0.21 | 0.04 | 0.85 | 0.15 | 89 |
| Irish | 155 | 0.66 | 0.30 | 0.04 | 0.81 | 0.19 | 6 |
| French | 316 | 0.66 | 0.30 | 0.04 | 0.81 | 0.19 | 6 |
| American | 160 | NA | NA | NA | 0.89 | 0.11 | 114 |
| American | 255 | 0.77 | 0.20 | 0.03 | 0.87 | 0.13 | 86 |
| American | 404 | NA | NA | NA | 0.82 | 0.18 | 115 |
| Canadian | 145 | 0.70 | 0.28 | 0.01 | 0.84 | 0.16 | 87 |
| Korean | 91 | 0.49 | 0.47 | 0.03 | 0.73 | 0.27 | 84 |
| Chinese | 125 | 0.49 | 0.42 | 0.10 | 0.70 | 0.30 | 83 |
| Hypertriglyceridemic | | | | | | | |
| British | 98 | 0.84 | 0.14 | 0.02 | 0.91 | 0.09 | 82 |
| British | 52 | 0.73 | 0.23 | 0.04 | 0.85 | 0.15 | 89 |
| Korean | 75 | 0.53 | 0.35 | 0.12 | 0.71 | 0.29 | 84 |
| Coronary artery disease | | | | | | | |
| British | 46 | 0.72 | 0.26 | 0.02 | 0.85 | 0.15 | 82 |
| British | 47 | 0.66 | 0.30 | 0.04 | 0.81 | 0.19 | 89 |
| Irish | 169 | 0.66 | 0.31 | 0.04 | 0.81 | 0.19 | 6 |
| French | 242 | 0.64 | 0.34 | 0.01 | 0.82 | 0.18 | 6 |
| American | 168 | 0.64 | 0.32 | 0.04 | 0.80 | 0.20 | 86 |
| American | 444 | NA | NA | NA | 0.83 | 0.17 | 115 |

Abbreviations: NA, not available.

of apoB-containing lipoproteins (44, 52, 60, 61, 96, 97), although not in all studies (43, 53). Overexpression of murine apoA-II in transgenic mice (46, 72, 98) or knocking out apoA-II also consistently caused increased or decreased plasma VLDL concentration, respectively (47, 73) (Table 1). Furthermore, the apoA-II-deficient mice presented decreased FFA, glucose, and insulin levels and increased remnant catabolism (47). On the other hand, murine apoA-II transgenic (developed with a genomic construction) mice had been shown to induce insulin resistance and increased adiposity when fed a 6% fat diet (99). Insulin resistance was characterized by normal fasting glucose levels and a 2-fold increase in insulin levels, concomitant with delayed plasma clearance of a glucose bolus and reduced muscle uptake of 2-deoxyglucose (99). Increased adiposity was probably due to a 50% decrease in triglyceride hydrolysis of transgenic adipose tissue (99). This insulin resistance and increased adiposity were not found in human apoA-II transgenic mice fed a high fat, high cholesterol cholate-containing diet (100), in double simian CETP/human apoA-II transgenic mice (61), or in apoE-deficient mice expressing human apoA-II (96). To date, we have found no signs of insulin resistance in human apoA-II transgenic mice fed a Western diet for 16 weeks (J. C. Escolà-Gil, J. Julve, C. Trocho, and F. Blanco-Vaca, unpublished results). This could be because a similar concentration of the transgenic protein leads to a much more important increase in plasma FFA in murine apoA-II than in the human counterpart (61, 72, 96, 99, 100), because it is likely that this increase in FFA is critical in the induction of insulin resistance (99). One of the reasons underlying this difference could be the displacement of mouse apoA-II seen in human apoA-II transgenic mice (44).

As stated previously, other studies exist that relate hypertriglyceridemia and increased FFA both in families enriched for CAD and in mice (90). In the latter there is, however, some degree of controversy as to whether the increase in triglycerides is found mainly in fed (97) or fasted animals (46, 61, 96, 99, 100). This also may imply differences in the mechanisms invoked to explain the origin of the hypertriglyceridemia. The report showing dramatic hypertriglyceridemia in human apoA-II transgenic mice fed a regular chow diet also demonstrated a significant increase in plasma human apoA-II in fed compared with fasted mice. In the former, their VLDL were a poor substrate for lipoprotein lipase and HL (97), an observation that has some precedents in human studies (32). However, our human apoA-II transgenic mice presented a considerable degree of fasting hypertriglyceridemia only when fed a high fat diet and autologous postheparin lipolytic activities were similar to those of the control mice. Furthermore, our apoA-II transgenic mice had a clearance of radioactively labeled triolein or oleyl ether VLDL and a concentration of human apoA-II in plasma that did not differ depending on fed or fasted status (96, 100). The hypertriglyceridemia in these apoA-II transgenic mice and apoE-deficient mice expressing human apoA-II was shown to be caused by increased VLDL triglyceride synthesis without changes in catabolism (96, 100). Double CETP/apoA-II transgenic mice induced a dramatic hyperlipidemia, especially when the animals were fed a high fat diet, by increasing VLDL triglyceride production (with re-

spect to both progenitors) and impairing VLDL triglyceride clearance (with respect to CETP transgenic mice) (61). In all cases, our transgenic mice presented concomitant increases in fasting FFA (61, 96, 100). We speculate that the differences in the mechanisms of hypertriglyceridemia between these two independently generated transgenic mice used in these studies (61, 96, 97, 100) are due to the fact that the 3-kb genomic human *apoA-II* injected into mice were not the same. One, ours, was obtained by digestion with *Msp*I (44), whereas the other was obtained by digestion with *Hin*dIII (97). This leaves our construction 260 bp longer at the 5′ end, but 174 bp shorter at the 3′ end, compared with that used by Boisfer et al. (97). As is shown in Fig. 1, the upstream *Hin*dIII cleavage site is located adjacent to the regulatory element AIIN. This could be of relevance in the regulation of human apoA-II synthesis in transgenic mice if the lack of sequences located upstream of the *Hin*dIII cleavage site influences the interaction of factors binding to the distal regulatory elements.

It is remarkable that in the two other reports of independently generated human apoA-II transgenic mice that did not present hypertriglyceridemia, the human *apoA-II* transgene construction contained the promoter of other genes (apoA-I and antithrombin III) or consisted of a 3-kb genomic construct prepared with *Hin*dIII (43, 53, 101). In the case of transgenic mice developed with the fusion product of a 3-kb fragment (which contained a 5′ end region and part of the first intron of the *apoA-I* gene fused with a 2-kb genomic construction of the human *apoA-II* gene cut with *Hin*dIII and containing its last three exons), the animals did not show hypertriglyceridemia when only 2-month-old males were studied under fasting conditions (43). However, these mice showed increased triglycerides when they were studied by other investigators under fed conditions at an age of 4–7 months (60).

Taking all these data together, and considering that an *apoA-II* intragenic marker excluded the *apoA-II* gene as the major familial combined hyperlipidemia locus found in chromosome 1q21-q23 (102), we propose that the *apoA-II* locus could be a modifier gene for familial combined hyperlipidemia. In this respect, it is worthy to note that apoA-II transgenic mice have been found to present several characteristics of the metabolic syndrome. One that has not been previously mentioned in this review is fatty liver (61, 100). Probably related to this capability of apoA-II to induce steatosis, the hepatitis C virus core protein, whose expression also produces steatosis, has been shown to bind apoA-II and, like apoA-II, its secretion was modulated by fibrate treatment (103).

## ROLE IN ATHEROSCLEROSIS SUSCEPTIBILITY

Although most studies show normal or decreased apoA-II levels in patients with CAD (104, 105), two Japanese patients from a single family with inherited apoA-II deficiency presented no clinical evidence of coronary atherosclerosis (5). In fact, several studies suggest that elevation of apoA-II may be proatherogenic (Table 1). In one study, elevated apoA-II-containing VLDL was related to the progression of CAD in patients treated with lovastatin (106), and in another the apoA-II/HDL cholesterol ratio was elevated in patients with this disease (107). Also, increased concentrations of apoA-II have been found in interstitial fluid of patients with myocardial infarction compared with those of controls (108).

Population studies of the apoA-II polymorphisms *Msp*I (6, 89, 86, 82, 115) and *Bst*NI (6) in control and CAD patients (Table 2) have found significant differences in genotype distribution only in one case (86). However, in this case there was no association of the *Msp*I polymorphism with lipid and apolipoprotein levels in the CAD population.

The development of transgenic mice expressing either human or murine apoA-II has provided novel insights into the role of apoA-II in atherosclerosis susceptibility. An early report showed that double human apoA-I/apoA-II transgenic mice lost part of the protection against atherosclerosis shown by human apoA-I transgenic mice fed a high fat atherogenic diet (109). In contrast, studies of mouse apoA-II transgenic mice revealed an increase in atherosclerosis susceptibility, even when the mice were fed a regular chow diet (98). This observation has been replicated and is fully consistent with the previous effects reported for natural variants of apoA-II synthesis in recombinant and congenic strains of mice (51, 72, 110). In addition, human apoA-II transgenic mice and double human apoA-II/CETP transgenic mice showed increased atherosclerosis susceptibility both when fed an atherogenic diet (71, 61) and when they were cross-bred with apoE-deficient mice and fed a regular chow diet (96). Only one study using independently generated human apoA-II transgenic mice showed decreased atherosclerosis susceptibility (53). Thus, the conclusion emerges that overexpression of apoA-II is proatherogenic and that, therefore, elevation of apoA-II is not a target for new drug therapies. The mechanisms of this proatherogenic capability of increased human apoA-II could be due to increased concentration of apoB-containing lipoprotein and decreased HDL cholesterol, impairment of reverse cholesterol transport due to decreased cholesterol efflux and esterification (44, 54, 55, 60, 67, 71, 96, 97, 100), as well as decreased protection toward LDL oxidation (51, 72) and insulin resistance (99). Therefore, apoA-II does not appear to play a protective role against atherosclerosis, and elevation of its concentration is likely to be harmful at least with respect to cardiovascular disease.

## CONCLUSIONS AND PERSPECTIVES

Analyses of genetically modified mice have, surprisingly, found a more consistent relationship between apoA-II and FFA and VLDL triglyceride metabolism than with HDL. These findings may be due in part to species-specific differences in the effect of mouse and human apoA-II on HDL. Nevertheless, the effects on triglyceride metabolism show enough consistency to postulate a function of apoA-II in the regulation of liver (which is a specu-

lation based on the main site of apoA-II expression) FFA and triglyceride metabolism. In this regard, a major aim for future research is to define this function in detail. To study liver gene expression of apoA-II transgenic and knockout mice and compare it with that of control mice could be worthwhile. Also of interest are the possible implications of these findings for human pathology. The study of apoA-II structure and expression could be of interest in at least three diseases: *1*) familial combined hyperlipidemia, in which *apoA-II* could be a modifier gene, that is, determines the degree of hypertriglyceridemia or development of metabolic syndrome; *2*) type 2 diabetes, in which its potential role as a susceptibility gene warrants further study; and *3*) in type V hyperlipidemia, a poorly known disease in which plasma apoA-II seems to be increased. Knowledge of whether this increased apoA-II concentration is a cause or an effect is necessary, especially considering that two types of predisposing conditions for type V hyperlipidemia, alcoholism and diabetes mellitus, could be associated with increased levels of apoA-II (99, 111, 112).

The role of apoA-II in HDL remains, despite many studies, unclear. It is apparent that increased apoA-II deteriorates two major antiatherogenic properties of HDL: reverse cholesterol transport and protection against LDL oxidative modification. Studies have begun to show that the presence of apoA-II in HDL may also be a determinant in receptor interaction and this could be of pathophysiological importance. In this regard, apoA-II interaction with Hsp60 and SR-BI is a topic of special interest. The *apoA-II* locus is an important genetic determinant of HDL cholesterol concentration. This may depend mainly on the synthesis of apoA-II; however, major gaps still remain to be filled in our understanding of apoA-II synthesis and catabolism.

The studies commented on in this review have clearly shown that raising human apoA-II does not raise HDL cholesterol or prevent atherosclerosis. Drugs that increase apoA-I, but not apoA-II, may be more powerful in the prevention of atherothrombotic cardiovascular disease. A better understanding of apoA-I and apoA-II gene regulation may be of help in the design of new and selective antiatherogenic drugs.

## NOTE ADDED IN PROOF

Yuhanna et al. (113) have reported that antibodies against apoA-I, but not antibodies against apoA-II, inhibited the HDL activation of endothelial nitric oxide synthase mediated through SR-BI binding.

This work was supported in part by grants SAF98-0097 and SAF99-0104, from the Ministerio de Ciencia y Tecnología, and 1999SGR00233, from the Comissionat per a Universitats i Recerca. We are also grateful to Christine O'Hara for excellent editorial assistance.

*Manuscript received 4 June 2001 and in revised form 25 June 2001.*

## REFERENCES

1. Li, W-H., M. Tanimura, C-C. Luo, S. Datta, and L. Chan. 1988. The apolipoprotein multigene family: biosynthesis, structure-function relationship, and evolution. *J. Lipid Res.* **29:** 245–271.
2. Lusis, A. J., B. A. Taylor, R. W. Wangenstein, and R. C. Leboeuf. 1983. Genetic control of lipid transport in mice. II. Genes controlling structure of high density lipoproteins. *J. Biol. Chem.* **258:** 5071–5078.
3. Weber, J. L., and P. E. May. 1989. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. *Am. J. Hum. Genet.* **44:** 388–396.
4. Knott, T. J., S. C. Wallis, M. E. Robertson, L. M. Priestley, M. Urdea, L. B. Rall, and J. Scott. 1985. The human apolipoprotein AII gene: structural organization and sites of expression. *Nucleic Acids Res.* **13:** 6387–6398.
5. Deeb, S. S., K. Takata, R. L. Peng, G. Kajiyama, and J. J. Albers. 1990. A splice-junction mutation responsible for familial apolipoprotein A-II deficiency. *Am. J. Hum. Genet.* **46:** 822–827.
6. Dupuy-Gorce, A. M., E. Desmarais, S. Vigneron, C. Buresi, V. Nicaud, A. Evans, G. Luc, D. Arveiler, P. Marques-Vidal, F. Cambien, L. Tiret, A. Crastes de Paulet, and G. Roizes. 1996. DNA polymorphisms in linkage disequilibrium at the 3′ end of the human APO AII gene: relationships with lipids, apolipoproteins and coronary heart disease. *Clin. Genet.* **50:** 191–198.
7. Scott, J., T. J. Knott, L. M. Priestley, M. E. Robertson, D. V. Mann, G. Kostner, G. J. Miller, and N. E. Miller. 1985. High-density lipoprotein composition is altered by a common DNA polymorphism adjacent to apoprotein AII gene in man. *Lancet.* **1:** 771–773
8. Hussain, M. M., and V. I. Zannis. 1990. Intracellular modification of human apolipoprotein (apoA-II) and sites of apoA-II synthesis: comparison of apoA-II with apoC-II and apoC-III isoproteins. *Biochemistry.* **29:** 209–217.
9. Zannis, V. I., H-Y. Kan, A. Kritis, E. Zanni, and D. Kardassis. 2001. Transcriptional regulation of the human apolipoprotein genes. *Front. Biosci.* **6:** d456–504.
10. Le Beyec, J., C. Benetollo, V. Chauffeton, A. Domiar, M-J. Lafreniere, J. Chambaz, P. Cardot, and A. D. Kalopissis. 1998. The −911/+29 sequence of human apolipoprotein A-II promoter is sufficient to confer liver restricted expression, developmental changes and response to a high fat diet in transgenic mice. *Transgenics.* **2:** 211–220.
11. Kardassis, D., M. Laccotripe, I. Talianidis, and V. I. Zannis. 1996. Transcriptional regulation of the genes involved in lipoprotein transport. The role of proximal promoters and long-range regulatory elements and factors in apolipoprotein gene region. *Hypertension.* **27:** 980–1008.
12. Chambaz, J., P. Cardot, D. Pastier, V. I. Zannis, and C. Claradas. 1991. Promoter elements and factors required for hepatic transcription of the human apoA-II gene. *J. Biol. Chem.* **266:** 11676–11685.
13. Cardot, P., J. Chambaz, C. Claradas, and V. I. Zannis. 1991. Regulation of the human apoA-II gene by the synergistic action of factors binding to the proximal and distal regulatory elements. *J. Biol. Chem.* **266:** 24460–24470.
14. Bossu, J-P., F. L. Chartier, N. Vu-Dac, J-C. Fruchart, and B. Laine. 1994. Transcription of the human apolipoprotein A-II is downregulated by the first intron of its gene. *Biochem. Biophys. Res. Commun.* **202:** 822–829.
15. Ribeiro, A., D. Pastier, D. Kardassis, J. Chambaz, and P. Cardot. 1999. Cooperative binding of upstream stimulatory factor and hepatic nuclear factor 4 drives the transcription of the human apolipoprotein A-II gene. *J. Biol. Chem.* **274:** 1216–1225.
16. Cardot, P., J. Chambaz, J. Kardassis, J. Chambaz, and V. I. Zannis. 1993. Factors participating in the liver-specific expression of the human apolipoprotein A-II gene and their significance for transcription. *Biochemistry.* **32:** 9080–9093.
17. Ladias, J. A. A., M. Hadzopoulou-Claradas, D. Kardassis, P. Cardot, J. Cheng, V. I. Zannis, and C. Claradas. 1992. Transcriptional regulation of human apolipoprotein genes apoB, apoCIII and apoAII by members of the steroid hormone receptor superfamily HNF4, ARP-1, EAR-2 and EAR-3. *J. Biol. Chem.* **267:** 15849–15860.
18. Vu-Dac, N., K. Schoonjans, V. Kosykh, J. Dallongeville, R. A. Heyman, B. Staels, and J. Auwerx. 1996. Retinoids increase human apolipoprotein A-II expression through activation of the retinoid X receptor but not the retinoic acid receptor. *Mol. Cell. Biol.* **16:** 3350–3360.

19. Vu-Dac, N., K. Schoonjans, V. Kosykh, J. Dallongeville, J-C. Fruchart, and B. Staels. 1995. Fibrates increase human apolipoprotein A-II expression through activation of the peroxisome proliferator-activated receptor. *J. Clin. Invest.* **96:** 741–750.
20. Pissios, P., H-Y. Kan, S. Nagaoka, and V. I. Zannis. 1999. SREBP-1 binds to multiple sites and transactivates the human apoA-II promoter in vitro: SREBP-1 mutants defective in DNA binding or transcriptional activation repress the apoA-II promoter activity. *Arterioscler. Thromb. Vasc. Biol.* **19:** 1456–1469.
21. Kan, H-Y., P. Pissios, J. Chambaz, and V. I. Zannis. 1999. DNA binding specificity and transactivation properties of SREBP-2 bound to multiple sites on the human apoA-II promoter. *Nucleic Acids Res.* **27:** 1104–1117.
22. Ikewaki, K., L. A. Zech, M. Kindt, H. B. Brewer, Jr., and D. J. Rader. 1995. Apolipoprotein A-II production rate is a major factor regulating the distribution of apolipoprotein A-I among HDL subclasses LpA-I and LpA-I:A-II in normolipidemic humans. *Arterioscler. Thromb. Vasc. Biol.* **15:** 306–312.
23. Shih, D. Q., H. M. Dansky, M. Fleisher, G. Assmann, S. S. Fajans, and M. Stoffel. 2000. Genotype/phenotype is associated with reduced apolipoprotein(AII), apolipoprotein(CIII), lipoprotein(a), and triglyceride levels. *Diabetes.* **49:** 832–837.
24. Panduro, A., Y-C. Lin-Lee, L. Chan, and D. A. Shafritz. 1990. Transcriptional and posttranscriptional regulation of apolipoprotein E, A-I, and A-II gene expression in normal rat liver and during several pathophysiologic states. *Biochemistry.* **29:** 8430–8435.
25. Brewer, H. B., S. E. Lux, R. Ronan, and K. M. John. 1972. Amino acid sequence of human apoLp-GlnII (apoA-II), an apolipoprotein isolated from the high density lipoprotein. *Proc. Natl. Acad. Sci. USA.* **69:** 1304–1308.
26. Weisgraber, K. H., and R. W. Mahley. 1978. Apoprotein (E-A-II) complex of human plasma lipoproteins. Characterization of this mixed disulfide and its identification in a high density lipoprotein subfraction. *J. Biol. Chem.* **253:** 6281–6288.
27. Borghini, I., R. W. James, M-C. Blatter, and D. Pometta. 1991. Distribution of apolipoprotein E between the free and apoA-II complexed forms in very-low- and high-density lipoproteins: functionl implications. *Biochim. Biophys. Acta.* **1083:** 139–146.
28. Blanco-Vaca, F., D. P. Via, C-y. Yang, J. B. Massey, and H. J. Pownall. 1992. Characterization of disulfide-linked heterodimers containing apolipoprotein D in human plasma lipoproteins. *J. Lipid Res.* **33:** 1785–1796.
29. Segrest, J. P., M. K. Jones, H. de Loof, C. G. Brouillette, Y. V. Venkatachalapathi, and G. N. Anantharamaiah. 1992. The amphipathic helix in the exchangable apolipoproteins: a review of secondary structure and function. *J. Lipid Res.* **33:** 141–166.
30. Remaley, A. T., A. W. Wong, U. K. Schumacher, M. S. Meng, H. B. Brewer, Jr., and J. M. Hoeg. 1993. O-linked glycosylation modifies the association of apolipoprotein A-II to high density lipoproteins. *J. Biol. Chem.* **268:** 6785–6790.
31. Bu, X., C. H. Warden, Y-R. Xia, C. De Meester, D. L. Puppione, S. Teruya, B. Lokensgard, S. Daneshmand, J. Brown, R. J. Gray, J. I. Rotter, and A. J. Lusis. 1994. Linkage analysis of the genetic determinants of high density lipoprotein concentrations and composition: evidence for involvement of the apolipoprotein A-II and cholesteryl ester transfer protein loci. *Hum. Genet.* **93:** 639–648.
32. Alaupovic, P., C. Knight-Gibson, C-S. Wang, D. Downs, E. Koren, H. B. Brewer, Jr., and R. E. Gregg. 1991. Isolation and characterization of an apoA-II-containing lipoprotein (LP-A-II-B complex) from plasma very low density lipoproteins of patients with Tangier disease and type V hyperlipoproteinemia. *J. Lipid Res.* **32:** 9–19.
33. Fruchart, J. C., and G. Aihaud. 1992. Apolipoprotein A-containing lipoprotein particles: physiological role, quantification, and clinical significance. *Clin. Chem.* **38:** 793–797.
34. Bekaert, E. D., P. Alaupovic, C. Knight-Gibson, R. A. Norum, M. J. Laux, and M. Ayrault-Jarrier. 1992. Isolation and partial characterization of lipoprotein A-II (LP A-II) particles of human plasma. *Biochim. Biophys. Acta.* **1126:** 105–113.
35. Doolittle, M. H., R. LeBoeuf, C. H. Warden, L. M. Bee, and A. J. Lusis. 1990. A polymorphism affecting apolipoprotein A-II translational efficiency determines high density lipoprotein size and composition. *J. Biol. Chem.* **265:** 16380–16388.
36. Clay, M. A., D. Pyle, K-A. Rye, and P. J. Barter. 2000. Formation of spherical, reconstituted high density lipoproteins containing both apolipoproteins A-I and A-II is mediated by lecithin:cholesterol acyltransferase. *J. Biol. Chem.* **275:** 9019–9025.
37. Chapman, M. J. 1986. Comparative analysis of mammalian plasma lipoproteins. *Methods Enzymol.* **128:** 70–143.
38. Gong, E. L., L. J. Stoltzfus, C. M. Brion, D. Murugesh, and E. M. Rubin. 1996. Contrasting in vivo effects of murine and human apolipoprotein A-II. *J. Biol. Chem.* **271:** 5984–5987.
39. Miller, C. G., T. D. Lee, R. LeBoeuf, and J. E. Shively. 1987. Primary structure of apolipoprotein A-II from inbred mouse strain BALB/c. *J. Lipid Res.* **28:** 311–319.
40. Scanu, A., P. Lagocki, and J. Chung. 1980. Effect of apolipoprotein A-II on the structure of high density lipoproteins: relationship to the activity of lecithin:cholesterol acyltransferase. *Ann. N.Y. Acad. Sci.* **348:** 160–173.
41. Labeur, C., G. Lambert, T. van Cauteren, N. Duverger, B. Vanloo, J. Chambaz, J. Vandekerckhove, G. Castro, and M. Rosseneu. 1998. Displacement of apoA-I from HDL by apoA-II or its C-terminal helix promotes the formation of pre-β1 migrating particles and decreases LCAT activation. *Atherosclerosis.* **139:** 351–362.
42. Durbin, D. M., and A. Jonas. 1999. Lipid-free apolipoproteins A-I and A-II promote remodelling of reconstituted high density lipoproteins and alter their reactivity with lecithin:cholesterol acyltransferase. *J. Lipid Res.* **40:** 2293–2302.
43. Schultz, J. R., E. L. Gong, M. R. McCall, A. V. Nichols, S. M. Clift, and E. M. Rubin. 1992. Expression of human apolipoprotein A-II and its effect on high density lipoproteins in transgenic mice. *J. Biol. Chem.* **267:** 21630–21636.
44. Marzal-Casacuberta, À., F. Blanco-Vaca, B. Y. Ishida, J. Julve-Gil, S. Shen, S. Calvet-Márquez, F. González-Sastre, and L. Chan. 1996. Functional lecithin:cholesterol acyltransferase deficiency and high density lipoprotein deficiency in transgenic mice overexpressing human apolipoprotein A-II. *J. Biol. Chem.* **271:** 6720–6728.
45. Rubin, E., B. Y. Ishida, S. M. Clift, and R. M. Krauss. 1991. Expression of human apolipoprotein A-I in transgenic mice results in reduced plasma levels of murine apolipoprotein A-I and the appearance of two new high density lipoprotein size subclasses. *Proc. Natl. Acad. Sci. USA.* **88:** 434–438.
46. Hedrick, C. C., L. W. Castellani, C. H. Warden, D. L. Puppione, and A. J. Lusis. 1993. Influence of mouse apolipoprotein A-II on plasma lipoproteins in transgenic mice. *J. Biol. Chem.* **268:** 20676–20682.
47. Weng, W., and J. L. Breslow. 1996. Dramatically decreased high density lipoprotein cholesterol increased remnant clearance, and insulin hypersensitivity in apolipoprotein A-II knockout mice suggest a complex role for apolipoprotein A-II in atherosclerosis susceptibility. *Proc. Natl. Acad. Sci. USA.* **93:** 14788–14794.
48. Rothblat, G. H., M. de la Llera-Moya, V. Atger, G. Kellner-Weibel, D. L. Williams, and M. C. Philips. 1999. Cell cholesterol efflux: integration of old and new observations provides new insights. *J. Lipid Res.* **40:** 781–796.
49. Fielding, C. J., and P. E. Fielding. 1995. Molecular physiology of reverse cholesterol transport. *J. Lipid Res.* **36:** 211–228.
50. Forte, T. M., J. K. Bielicki, R. Goth-Goldstein, J. Selmek, and M. R. McCall. 1995. Recruitment of cell phospholipids and cholesterol by apolipoproteins A-II and A-I: formation of nascent apolipoprotein-specific HDL that differ in size, phospholipid composition, and reactivity with LCAT. *J. Lipid Res.* **36:** 148–157.
51. Castellani, L. W., M. Navab, B. J. van Lenten, C. C. Hedrick, S. Y. Hama, A. M. Goto, A. M. Fogelman, and A. J. Lusis. 1997. Overexpression of apolipoprotein A-II in transgenic mice converts high density lipoproteins to proinflammatory particles. *J. Clin. Invest.* **100:** 464–474.
52. Julve-Gil, J., E. Ruiz-Pérez, R. P. Casaroli-Marano, A. Marzal-Casacuberta, J. C. Escolà-Gil, F. González-Sastre, and F. Blanco-Vaca. 1999. Free cholesterol deposition in the cornea of human apolipoprotein A-II transgenic mice with functional lecithin:cholesterol acyltransferase deficiency. *Metabolism.* **48:** 415–421.
53. Tailleux, A., M. Bouly, G. Luc, G. Castro, J. M. Caillaud, N. Hennuyer, P. Poulain, J. C. Fruchart, N. Duverger, and C. Fievet. 2000. Decreased susceptibility to diet-induced atherosclerosis in human apolipoprotein A-II transgenic mice. *Arterioscler. Thromb. Vasc. Biol.* **20:** 2453–2458.
54. Castro, G., L. P. Nihoul, C. Dengremont, C. de Geitere, B. Delfly, A. Tailleux, C. Fievet, N. Duverger, P. Denèfle, J-C. Fruchart, and E. M. Rubin. 1997. Cholesterol efflux, lecithin:cholesterol acyltransferase activity, and pre-beta particle formation by serum from human apolipoprotein A-I/apolipoprotein A-II transgenic

mice consistent with the latter being less effective for reverse cholesterol transport. *Biochemistry.* **36:** 2243–2249.
55. Chiesa, G., C. Parolini, M. Canavesi, N. Colombo, C. R. Sirtori, R. Fumagalli, G. Franceschini, and F. Bernini. 1998. Human apolipoproteins A-I and A-II in cell cholesterol efflux. Studies with transgenic mice. *Arterioscler. Thromb. Vasc. Biol.* **18:** 1417–1423.
56. Remaley, A. T., J. A. Stonik, S. J. Demosky, E. B. Neufeld, A. V. Bocharov, T. G. Vishnyakova, T. L. Eggerman, A. P. Patterson, N. Duverger, S. Santamarina-Fojo, and H. B. Brewer, Jr. 2001. Apolipoprotein specificity for lipid efflux by the human ABCAI transporter. *Biochem. Biophys. Res. Commun.* **280:** 818–823.
57. Lagrost, L., L. Perségol, C. Lallemant, and P. Gambert. 1994. Influence of apolipoprotein composition of high density lipoprotein particles on cholesteryl ester transfer protein activity. *J. Biol. Chem.* **269:** 3189–3197.
58. Rye, K-A., and P. J. Barter. 1994. The influence of apolipoproteins on the structure and function of spheroidal, reconstituted high density lipoproteins. *J. Biol. Chem.* **269:** 10298–10303.
59. Masson, D., N. Duverger, F. Emmanuel, and L. Lagrost. 1997. Differential interaction of the human cholesteryl ester transfer protein with plasma high density lipoproteins (HDLs) from humans, control mice, and transgenic mice to human HDL apolipoproteins. *J. Biol. Chem.* **272:** 24287–24293.
60. Zhong, S. B., I. J. Goldberg, C. Bruce, E. M. Rubin, J. L. Breslow, and A. R. Tall. 1994. Human apoA-II inhibits the hydrolysis of HDL triglycerides and the decrease of HDL size induced by hypertriglyceridemia and cholesteryl ester transfer protein in transgenic mice. *J. Clin. Invest.* **94:** 2457–2467.
61. Escolà-Gil, J. C., J. Julve-Gil, À. Marzal-Casacuberta, J. Ordóñez-Llanos, F. González-Sastre, and F. Blanco-Vaca. 2001. ApoA-II expression in CETP transgenic mice increases VLDL production and impairs VLDL clearance. *J. Lipid Res.* **42:** 241–248.
62. Pussinen, P. J., M. Jauhiainen, J. Metso, L. E. Pyle, Y. L. Marcel, N. H. Fidge, and C. Ehnholm. 1998. Binding of phospholipid transfer protein (PLTP) to apolipoproteins A-I and A-II: location of a PLTP binding domain in the amino terminal region of apoA-I. *J. Lipid Res.* **39:** 152–161.
63. Albers, J. J., G. Wolfbauer, M. C. Cheung, J. R. Day, A. F. T. Ching, S. Lok, and A-Y. Tu. 1995. Functional expression of human and mouse plasma phospholipid transfer protein: effect of recombinant and plasma PTLP on HDL subspecies. *Biochim. Biophys. Acta.* **1258:** 27–34.
64. Pussinen, P. J., M. Jauhiainen, and C. Ehnholm. 1997. ApoA-II/apoA-I molar ratio in the HDL particle influences phospholipid transfer protein-mediated HDL interconversion. *J. Lipid Res.* **38:** 12–21.
65. Durbin, D. M., and A. Jonas. 1997. The effect of apolipoprotein A-II on the structure and function of apolipoprotein A-I in a homogeneous reconstituted high density lipoprotein particle. *J. Biol. Chem.* **272:** 31333–31339.
66. Leroy, A., J. Dallongeville, and J-C. Fruchart. 1995. Apolipoprotein A-I-containing lipoproteins and atherosclerosis. *Curr. Opin. Lipidol.* **6:** 281–285.
67. Francone, O. L., E. L. Gong, D. S. Ng, C. J. Fielding, and E. M. Rubin. 1995. Expression of human lecithin:cholesterol acyltransferase in transgenic mice: effect of human apolipoprotein AI and human apolipoprotein AII on plasma lipoprotein cholesterol metabolism. *J. Clin. Invest.* **96:** 1440–1448.
68. Thuren, T. Hepatic lipase and HDL metabolism. 2000. *Curr. Opin. Lipidol.* **11:** 277–283.
69. Hime, N. J., P. J. Barter, and K-A. Rye. 1998. The influence of apolipoproteins on the hepatic lipase-mediated hydrolysis of high density lipoprotein phospholipid and triglyceride. *J. Biol. Chem.* **273:** 27191–27198.
70. Hime, N. J., P. J. Barter, and K. A. Rye. 2001. Evidence that apolipoprotein A-I facilitates hepatic lipase-mediated phospholipid hydrolysis in reconstituted HDL containing apolipoprotein A-II. *Biochemistry.* **40:** 5496–5505.
71. Escolà-Gil, J. C., À. Marzal-Casacuberta, J. Julve-Gil, B. Y. Ishida, J. Ordóñez-Llanos, L. Chan, F. González-Sastre, and F. Blanco-Vaca. 1998. Human apolipoprotein A-II is a pro-atherogenic molecule when it is expressed in transgenic mice at a level similar to that in humans: evidence of a potentially relevant species-specific interaction with diet. *J. Lipid Res.* **39:** 457–462.
72. Hedrick, C. C., L. W. Castellani, H. Wong, and A. J. Lusis. 2001. In vivo interactions of apoA-II, apoA-I, and hepatic lipase contributing to HDL structure and antiatherogenic functions. *J. Lipid Res.* **42:** 563–570.
73. Weng, W., N. A. Brandenburg, S. B. Zhong, J. Halkias, L. Wu, X-C. Jiang, A. Tall, and J. L. Breslow. 1999. ApoA-II maintains HDL levels in part by inhibition of hepatic lipase: studies in apoA-II and hepatic lipase double knockout mice. *J. Lipid Res.* **40:** 1064–1070.
74. Mowry, H-O., W. Patsch, L. C. Smith, A. M. Gotto, Jr., and J. R. Patsch. 1992. Different reactivities of high density lipoprotein 2 subfractions with hepatic lipase. *J. Lipid Res.* **33:** 1269–1279.
75. Fidge, N. H. 1999. High density lipoprotein receptors, binding proteins, and ligands. *J. Lipid Res.* **40:** 187–201.
76. Rinninger, F., T. Kaiser, E. Windler, H. Greten, J-C. Fruchart, and G. Castro. 1998. Selective uptake of cholesteryl esters from high-density lipoprotein derived LpA-I and LpA-I:A-II particles by hepatic cells in culture. *Biochim. Biophys. Acta.* **1393:** 277–291.
77. Pilon, A., O. Briand, S. Lestavel, C. Copin, Z. Majd, J-C. Fruchart, G. Castro, and V. Clavey. 2000. Apolipoprotein AII enrichment of HDL enhances their affinity for class B type I scavenger receptor but inhibits specific cholesteryl ester uptake. *Arterioscler. Thromb. Vasc. Biol.* **20:** 1074–1081.
78. De Beer, M. C., D. M. Durbin, L. Cai, N. Mirocha, A. Jonas, N. R. Webb, F. C. de Beer, and D. R. van der Westhuyzen. 2001. Apolipoprotein A-II modulates the binding and selective uptake of reconstituted HDL by scavenger receptor BI. *J. Biol. Chem.* **276:** 15832–15839.
79. Moestrup, S. K., and R. Kozyraki. 2000. Cubilin, a high-density lipoprotein receptor. *Curr. Opin. Lipidol.* **11:** 133–140.
80. Bocharov, A. V., T. G. Vishnyakova, I. N. Baranova, A. T. Remaley, A. P. Patterson, and T. L. Eggerman. 2000. Heat shock protein 60 is a high-affinity high-density lipoprotein binding protein. *Biochem. Biophys. Res. Commun.* **277:** 228–235.
81. Bonnefont-Rousselot, D., P. Thérond, J-L. Beaudeux, J. Peynet, A. Legrand, and J. Delattre. 1999. High density lipoproteins (HDL) and the oxidative hypothesis of atherosclerosis. *Clin. Chem. Lab. Med.* **37:** 939–948.
82. Ferns, G. A. A., C. S. Sheley, J. Stocks, A. Rees, H. Paul, F. Baralle, and D. J. Galton. 1986. A DNA polymorphism of the apolipoprotein A-II gene. *Hum. Genet.* **74:** 302–306.
83. Saha, N., J. A. M. A. Tan, and J. S. H. Tay. 1992. MspI polymorphism of the apolipoprotein A-II gene, serum lipids, and apolipoproteins in Chinese from Singapore. *Hum. Hered.* **42:** 293–297.
84. Hong, S. H., B. Y. Kang, W. H. Park, J. Q. Kim, and C. C. Lee. 1998. Association between apolipoprotein A2 MspI polymorphism and hypertriglyceridemia in Koreans. *Hum. Biol.* **70:** 41–46.
85. Rajput-Williams, J., J. Eyre, M. N. Nanjee, D. Crook, J. Scott, and N. E. Miller. 1989. Plasma lipoprotein lipids in relation to the MspI polymorphism of the apolipoprotein A-II gene in Caucasian men. *Atherosclerosis.* **77:** 31–36.
86. Civeira, F., J. Genest, M. Pocovi, D. N. Salem, P. N. Herbert, P. W. F. Wilson, E. J. Schaefer, and J. M. Ordovas. 1992. The *MspI* restriction fragment length polymorphism 3′ to the apolipoprotein gene: relationship with lipids, apolipoproteins, and premature coronary artery disease. *Atherosclerosis.* **92:** 165–176.
87. Vohl, M-C., B. Lamarche, J. Bergeron, S. Moorjani, D. Prud'homme, A. Nadeau, A. Tremblay, P. J. Lupien, C. Bouchard, and J-P. Després. 1997. The *Msp* I polymorphism of the apolipoprotein A-II gene as a modulator of the dyslipidemic state found in visceral obesity. *Atherosclerosis.* **128:** 183–190.
88. Myklebost, O., S. Rogne, I. Hjermann, B. Olaisen, and H. Prydz. 1990. Association analysis of lipid levels and apolipoprotein restriction fragment length polymorphisms. *Hum. Genet.* **86:** 209–214.
89. Thorn, J. A., J. Stocks, D. Reichl, J. C. Alcolado, J. C. Chamberlain, and D. Galton. 1993. Variability of plasma apolipoprotein (apo) A-II levels associated with an apoA-II gene polymorphism in monozygotic twin pairs. *Biochim. Biophys. Acta.* **1180:** 299–303.
90. Warden, C. H., A. Daluiski, X. Bu, D. A. Purcell-Huynh, C. De Meester, D. L. Puppione, S. Teruya, B. Lokensgard, S. Daneshman, J. Brown, R. J. Gray, J. I. Rotter, and A. J. Lusis. 1993. Evidence for linkage of the apolipoprotein A-II locus to plasma apolipoprotein A-II and free fatty acid levels in mice and humans. *Proc. Natl. Acad. Sci. USA.* **90:** 10886–10890.
91. Hannis, C. L., R. Chakraborty, and D. Hewett-Emmett. 1985. How much of variability in apolipoprotein A-II concentration is explained by polymorphism adjacent to apoA-II gene? *Lancet.* **1:** 1339–1340.

92. Magill, P., S. N. Rao, N. E. Miller, A. Nicoll, J. Brunzell, J. St. Hilaire, and B. Lewis. 1982. Relationships between the metabolism of high density and very-low density lipoproteins in man: studies of apolipoprotein kinetics and adipose tissue lipoprotein lipase activity. *Eur. J. Clin. Invest.* **12:** 113–120.
93. Vionnet, N., E. H. Hani, S. Dupont, S. Gallina, S. Francke, S. Dotte, F. De Matos, F. Durand, F. Lepetre, C. Lecoeur, P. Gallina, L. Zekiri, C. Dina, and P. Froguel. 2000. Genomewide search for type 2 diabetes-susceptibility genes in French whites: evidence for a novel susceptibility locus for early onset diabetes on chromosome 3q27-qter and independent replication of a type 2-diabetes locus on chromosome 1q21-q24. *Am. J. Hum. Genet.* **67:** 1470–1480.
94. Elbein, S. C., M. D. Hoffman, K. Teng, M. F. Leppert, and S. J. Hasstedt. 1999. A genome-wide search for type 2 susceptibility gene in Utah Caucasians. *Diabetes.* **48:** 1175–1182.
95. Hanson, R. L., M. G. Ehm, D. J. Pettitt, M. Prochazka, D. B. Thompson, D. Timberlake, T. Foroud, S. Kobes, L. Baier, D. K. Burns, L. Almasy, J. Blangero, W. T. Garvey, P. H. Bennet, and W. C. Knowler. 1998. An autosomal genomic scan for loci linked to type II diabetes mellitus and body-mass index in Pima Indians. *Am. J. Hum. Genet.* **63:** 1130–1138.
96. Escolà-Gil, J. C., J. Julve, À. Marzal-Casacuberta, J. Ordóñez-Llanos, F. González-Sastre, and F. Blanco-Vaca. 2000. Expression of human apolipoprotein A-II in apolipoprotein E-deficient mice induces features of familial combined hyperlipidemia. *J. Lipid Res.* **41:** 1328–1338.
97. Boisfer, E., G. Lambert, V. Atger, N. Q. Tran, D. Pastier, C. Benetollo, J-F. Trottier, I. Beaucamps, M. Antonucci, M. Laplaud, S. Griglio, J. Chambaz, and A-D. Kalopissis. 1999. Overexpression of human apolipoprotein A-II induces hypertriglyceridemia due to defective very low density lipoprotein hydrolysis. *J. Biol. Chem.* **274:** 11564–11572.
98. Warden, C. H., C. C. Hedrick, J-H. Qiao, L. W. Castellani, and A. J. Lusis. 1993. Atherosclerosis in transgenic mice overexpressing apolipoprotein A-II. *Science.* **261:** 469–471.
99. Castellani, L. W., A. M. Goto, and A. J. Lusis. 2001. Studies with transgenic mice indicate a role for HDLs in adiposity and insulin resistance. *Diabetes.* **50:** 643–651.
100. Julve, J., J. C. Escolà-Gil, À. Marzal-Casacuberta, J. Ordóñez-Llanos, F. González-Sastre, and F. Blanco-Vaca. 2000. Increased production of very-low-density lipoproteins in transgenic mice overexpressing human apolipoprotein A-II and fed with a high-fat diet. *Biochim. Byophys. Acta.* **1488:** 233–244.
101. Tremp, G. L., N. Duchange, D. Branellec, S. Cereghini, A. Tailleux, L. Berthou, C. Fievet, N. Touchet, B. Schombert, J-C. Fruchart, M. M. Zakin, and P. Denèfle. 1995. A 700-bp fragment of the human antithrombin III promoter is sufficient to confer high, tissue-specific expression on human apolipoprotein A-II in transgenic mice. *Gene.* **156:** 199–205.
102. Pajukanta, P., I. Nuotio, J. D. Terwilliger, K. V. K. Porkka, K. Ylitalo, J. Pihlajamäki, A. J. Suomalainen, A-C. Syvänen, T. Lehtimäki, J. S. A. Viikari, M. Laakso, M-R. Taskinen, C. Ehnholm, and L. Peltonen. 1998. Linkage of familial combined hyperlipidaemia to chromosome 1q21-q23. *Nat. Genet.* **18:** 369–373.
103. Sabile, A., G. Perlemuter, F. Bono, K. Kohara, F. Demauge, M. Kohara, Y. Matsuura, T. Miyamura, C. Brechot, and G. Barba. 1999. Hepatitis C virus core protein binds to apolipoprotein AII and its secretion is modulated by fibrates. *Hepatology.* **30:** 1064–1076.
104. Buring, J. E., G. T. O'Connor, S. Z. Goldhaber, B. Roser, P. N. Herbert, C. B. Blum, J. L. Breslow, and C. H. Hennekens. 1992. Decreased HDL2 and HDL3 cholesterol, apo A-I and apo A-II, and increased risk of myocardial infarction. *Circulation.* **85:** 22–29.
105. O'Brien, T., T. T. Nguyen, B. J. Hallaway, D. Hodge, K. Bailey, D. Holmes, and B. A. Kotke. 1995. The role of lipoprotein A-I and lipoprotein A-I/A-II in predicting coronary artery disease. *Arterioscler. Thromb. Vasc. Biol.* **15:** 228–231.
106. Alaupovic, P., W. J. Mack, C. Knight-Gibson, and H. N. Hodis. 1997. The role of triglyceride-rich lipoprotein families in the progression of atherosclerotic lesions as determined by sequential coronary angiography from a controlled clinical trial. *Arterioscler. Thromb. Vasc. Biol.* **17:** 715–722.
107. Kawagushi, A., Y. Miyao, T. Noguchi, H. Nonogi, M. Yamagishi, K. Miyatake, Y. Kamikubo, K. Kumeda, M. Tsushima, A. Yamamoto, and H. Kato. 2000. Intravascular free tissue factor pathway inhibitor is inversely correlated with HDL cholesterol and postheparin lipoprotein lipase but proportional to apolipoprotein A-II. *Arterioscler. Thromb. Vasc. Biol.* **20:** 251–258.
108. Luc, G., Z. Majd, P. Poulain, L. Elkhalil, and J-C. Fruchart. 1996. Interstitial fluid apolipoprotein A-II: an association with the occurrence of myocardial infarction. *Atherosclerosis.* **127:** 131–137.
109. Schultz, J. R., J. G. Verstuyft, E. L. Gong, A. V. Nichols, and E. M. Rubin. 1993. Protein composition determines the anti-atherogenic properties of HDL in transgenic mice. *Nature.* **365:** 762–764.
110. Mehrabian, M., J-H. Qiao, R. Hyman, D. Ruddle, C. Laughton, and A. J. Lusis. 1993. Influence of the apoA-II locus on HDL levels and fatty acid streak development in mice. *Arterioscler. Thromb. Vasc. Biol.* **13:** 1–10.
111. Gottrand, F., L. Beghin, N. Duhal, B. Lacroix, J-P. Bonte, J. C. Fruchart, and G. Luc. 1999. Moderate red wine consumption in healthy volunteers reduced plasma clearance of apolipoprotein AII. *Eur. J. Clin. Invest.* **29:** 387–394.
112. De Oliveira, E., E. R. Silva, D. Foster, M. McGee Harper, C. E. Seidman, J. D. Smith, J. L. Breslow, and E. A. Brinton. 2000. Alcohol consumption raises HDL cholesterol levels by increasing the transport rate of apolipoproteins A-I and A-II. *Circulation.* **102:** 2347–2352.
113. Yuhanna, I. S., Y. Zhu, B. E. Cox, L. D. Hahner, S. Osborne-Lawrence, P. Lu, Y. L. Marcel, R. G. W. Anderson, M. E. Mendelsohn, H. H. Hobbs, and P. W. Shaul. 2001. High-density lipoprotein binding to scavenger-BI activates endothelial nitric oxide synthase. *Nature.* **7:** 853–857.