Apolipoprotein(a) Gene Enhancer Resides within a LINE Element*

Zhuoying Yang, Dario Boffelli, Nataya Boonmark, Karen Schwartz, and Richard Lawn†‡

From the Falk Cardiovascular Research Center, Stanford University School of Medicine, Stanford, California 94305-5246

Apolipoprotein(a), (apo(a)), is the distinguishing protein portion of the lipoprotein(a) particle, elevated plasma levels of which are a major risk factor for cardiovascular disease. A search for enhancer elements that control the transcription of the apo(a) gene led to the identification of an upstream element that contains target binding sites for members of the Ets and Sp1 nuclear protein families. The enhancer element functions in either orientation to confer a greater than 10-fold increase in the activity of the apo(a) minimal promoter in cultured hepatocyte cells. Unexpectedly, the enhancer element is located within a LINE retrotransposon element, suggesting that LINE elements may function as mobile regulatory elements to control the expression of nearby genes.

Elevated levels of lipoprotein(a) (Lp(a))† are a major risk factor for atherosclerosis, myocardial infarction, and cerebrovascular stroke (see Refs. 1 and 2 and references therein). Lp(a) is a lipoprotein consisting of low density lipoprotein to which is covalently linked a unique protein, apolipoprotein(a) (apo(a)) (3, 4). Apo(a) is a large, highly polymorphic glycoprotein with sequence homology to plasminogen. Depending on individual alleles, it contains from 12 to 50 copies of a domain which resembles kringle four of plasminogen, plus a single kringle five-like domain, and an inactive protease domain (5, 6). When plasminogen is activated to plasmin, it can cleave fibrin and several other protein substrates. By competing with plasminogen for fibrin binding through its apo(a) component, Lp(a) can reduce the conversion of plasminogen to the active plasmin and inhibit clot lysis (7–9). By a similar mechanism, Lp(a) can reduce plasmin-dependent activation of latent transforming growth factor-β, resulting in smooth muscle cell activation, lipid accumulation, and inflammatory damage to the endothelium (10, 11). Transgenic mice expressing the human apo(a) gene are more susceptible to the development of fatty streak-type lesions when fed a high fat diet, and they exhibit prolonged fibrinolysis (12, 13).

The concentration of Lp(a) in human plasma varies about 1000-fold in the population and is inherited in a Mendelian co-dominant fashion. Roughly 97% of this variation maps to the locus of the apo(a) gene (14–16). Hence, the regulation of apo(a) gene expression is significant in controlling pathological plasma levels of Lp(a). About one half of the large variation of apo(a) plasma concentration is due to transcription efficiency, and one half to the post-transcriptional effects of size polymorphism (16). Since isoforms of apo(a) with greater numbers of kringle domains transit through the cell less rapidly and efficiently, there is a rough inverse correlation between size of apo(a) isoforms and plasma concentration (17, 18). However, even within alleles matched for isoform size, there exists a greater than 200-fold range of plasma concentration (19–22). Transcription control is a likely cause of this variation. Numerous studies have found a large variation in apo(a) mRNA quantity in humans and primates, which corresponds with plasma concentration in most cases (23–26). We have previously shown that the liver-specific expression of the apo(a) gene is partly mediated by the apo(a) proximal promoter, which is located from −98 to +130 relative to its transcription start site and responds to the liver-enriched transcription factor HNF-1α (27). However, there are several indications that additional transcriptional regulatory elements must contribute to the high levels of apo(a) gene expression: activity of this core promoter is relatively weak in in vitro transfection assays, it does not account for the sex hormone responsiveness of the apo(a) gene, and it contains far less sequence polymorphism than could account for the inherited variation in gene expression, which is known to map to this locus. A further indication that regulatory sequences exist further 5’ to the apo(a) gene comes from transgenic mouse experiments. Deletion of the region 5’ to the apo(a) promoter from a yeast artificial chromosome severely compromises apo(a) expression in transgenic mice compared with a yeast artificial chromosome containing approximately 80 kb of upstream sequence.2

The apo(a) gene is part of a cluster of genes and pseudogenes on chromosome six which share over 70% sequence identity (28, 29). The apo(a) and the plasminogen genes are organized in a head to head configuration separated by about 40 kb. We have systematically tested this entire intergenic region for additional apo(a) regulatory elements. In this study, we describe an apo(a) transcription control region (ACR) that is located 20 kb 5’ to the apo(a) transcription start site. The enhancer region contains an essential element responding to the members of the Ets transcription factor family. In addition, the binding of Sp1 as well as several other transcription factors around the Ets site also contributes to maximal enhancer activity. Unexpectedly, the apo(a) enhancer region resides within a LINE element. LINE 1 (L1) elements are long interspersed repeat elements that number from 50,000 to 100,000 in the human genome. Although greater than 90% of them are truncated, full-length L1s are approximately 6 kb in length and contain a 5’-untranslated region with internal transcription regulatory

---

* This research was supported in part by National Institutes of Health Program Project Grant 48638 and post-doctoral fellowships from the National Institutes of Health, American Heart Association California Affiliate, and Swiss National Science Foundation. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom correspondence should be addressed: Falk Cardiovascular Research Center, Stanford University School of Medicine, 300 Pasteur Drive, Stanford CA 94305-5246. Tel.: 650-725-4494; Fax: 650-725-1599; E-mail: richard.lawn@forsythe.stanford.edu.

‡ This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: Lp(a), lipoprotein(a); apo(a), apolipoprotein(a); HNF, hepatocyte nuclear factor; kb, kilobase pair(s); bp, base pair(s); PCR, polymerase chain reaction; ACR, apo(a) gene transcription control region; L1, LINE element 1; BAC, bacterial artificial chromosome.

2 S. Hughes and E. Rubin, personal communication.
elements, two open reading frames, and a 3'-untranslated region that terminates in a poly(A) tail (30–32). This is the first report of an L1 element affecting the expression of a flanking gene. In contrast, the corresponding regions from 10 other randomly selected human L1 elements were found to have reduced enhancer activity as well as one or more nucleotide differences in the core Ets binding site sequence. Hence the L1 element upstream of the apo(a) gene may represent a small subset of these repeat elements that have the ability to alter the expression of neighboring genes.

MATERIALS AND METHODS

pGL3-1.4RI and pGL3a Plasmid Construction—Plasmid pGL3-1.4RI was constructed from pGLA1 (27), with subsequent manipulation to create a unique EcoRI site and to remove unnecessary polylinker sequences. It contains human apo(a) genomic 5′ sequences from an EcoRI site 1.4 kb upstream of the transcription start site to an MscI site 130 bp downstream of the start site, fused to the luciferase gene in pGL3-basic (Promega). pGL3a is a similar luciferase reporter plasmid containing a fragment of the 5′-genomic sequence used above. It contains the minimal promoter of apo(a) from −98 to +130 (27) and derives from pGL3-1.4RI by removal of an EcoRI-PvuII fragment and subsequent regeneration of the EcoRI site.

Bacterial Artificial Chromosome (BAC) Library Screening and Subcloning—A human genomic DNA library in the BAC plasmid (Genome Systems, Inc.) was screened with probes representing the 5′-flanking regions of apo(a) and plasminogen genes. One of the two positive clones (pBAC2), containing an insert of ~150 kb, was confirmed to have the entire intergenic region as well as portions of the coding regions of both apo(a) and plasminogen genes by Southern blot analysis with apo(a) and plasminogen cDNA probes.

EcoRI restriction fragments of pBAC2 DNA, as numbered in Fig. 1, were subcloned into Bluescript (Stratagen) either directly after digestion of pBAC2 or following PCR amplification. The pBAC2 DNA was also double-digested with SacI and SalI for the purpose of cloning fragments 8b–4 into Bluescript (Stratagen), taking advantage of the fact that there is only one SalI site in the entire pBAC2 insert. Fragments cloned into Bluescript were excised with EcoRI, SacI, or KpnI, and subcloned into pGL3a. In most cases, the fragments were initially tested in only one orientation relative to the transcription direction of luciferase based on the convenience of subcloning.

Cell Culture and Transfection Assays—Human hepatoma HepG2 cells and HeLa cells were maintained in Dulbecco’s modified medium supplemented with 10% fetal calf serum. 2 × 10⁵ cells were seeded in 12-well cell culture plates 24 h prior to transfection by a calcium phosphate co-precipitation method according to the manufacturer’s protocol (Promega) as we described previously (27). Transfections were done in triplicate. Briefly, 3 μg of pGL3-based luciferase expression plasmid and 1 μg of the control pSV-β-galactosidase (Promega) control plasmid, with or without 1 and 3 μg of Ets-1 expression plasmid were added to each well covered with 2 ml of culture medium. 48 h after transfection, the cells were harvested and lysed, and luciferase and β-galactosidase assays were performed using the Dual Light Kit (Tropix, Inc.) according to the protocol provided. To control for transfection efficiency, results are reported as the ratio of luciferase to β-galactosidase activity.

Deletion, Mutagenesis, and Diagnostic Fragments—Fragments of genomic DNA in reporter plasmids were released using available restriction sites. Specifically, construct nos. 3 and 6 (Fig. 2) were the result of removing a NheI fragment (one NheI site from the vector upstream of EcoRI, and the second one located at nucleotide 386 of L1, according to the L1 numbering system of Swergold (30)) from the L1 numbering system of Swergold (30)) from the L1 numbering system of Swergold (30) to remove unnecessary polylinker sequences. This fragment was generated with PCR primers L1-3 (5′-gatgaatctGCTAGCACACGAGCTTCTGA) and L1-4 (5′-ttggaacctAGTGCCTGCTTGGGAGA) to yield the enhancer-containing region corresponding to nucleotides 386–659 of L1 (lowercase letters denote added linker sequences).

Site-specific mutagenesis was carried out according to the mismatch PCR primer protocol (33) with oligonucleotides L1-3 and L1-4 as the

FIG. 1. Apo(a)/plasminogen genomic locus. The apo(a)/plasminogen locus includes apo(a)-arg-C, apo(a), and plasminogen genes and the apo(a)-arg-B pseudogene (28, 29). The genes are represented by shaded boxes with the direction of transcription indicated by arrows. The pBAC2 insert is indicated beneath. Below is a blowup of the region between the apo(a) and the plasminogen genes, with EcoRI restriction sites (+) and the position of the LINE (L1) element indicated with its predicted direction of transcription and "one reading frames” (shading) indicated. However, sequence analysis shows these canonical reading frames to be interrupted by numerous stop codons. Size scales are shown at top and bottom.
Apolipoprotein(a) Gene Enhancer

Fig. 2. Deletion analysis to define the enhancer region. Panel A, schematic representation of the luciferase expression plasmids. The test DNA fragment (filled bar) was ligated upstream of the apo(a) core promoter from nucleotide −98 to +130 (hashed bar) and luciferase cDNA. The thin line represents regions deleted. To allow reference to the restriction sites as indicated in construct no. 1, all deletion constructs are drawn in the same, positive orientation. However, constructs were either tested with these fragments ligated in the same (positive) or opposite orientation as indicated by a + or − sign in the column to the right. Panel B, luciferase activity in transfected HepG2 cells. The relative luciferase activity, normalized to β-galactosidase activity, is shown for each corresponding construct. The data represent the mean of three transfections plus the standard deviation. The activity of pGL3a, containing only the core promoter fragment (construct 0), was set to 100%.

Minimal Enhancer Region Is Defined by Deletion Analysis—To locate the enhancer elements in this region, it was subjected to deletion analysis. As shown in Fig. 2, construct no. 7, deletion of the KpnI-PvuII fragment at the 5′ end of the 1.8-kb SacI-KpnI fragment (orientation relative to the apo(a) gene) resulted in a total loss of the enhancer activity, indicating the presence of essential positive elements within this region. By itself, this KpnI-PvuII fragment is sufficient to confer an over-10-fold activation (Fig. 2, no. 8). A further truncation (no. 6) yields a fragment without activity. We postulated that the functional enhancer domain resides within the 274-nucleotide PvuI-PvuII fragment, which gives a greater than 10-fold activation when ligated in either orientation 5′ to the apo(a) core promoter and luciferase cDNA (Fig. 2, nos. 9 and 10). We designated this the apo(a) transcription control region (ACR). Deleting substantial amounts of the 1.8-kb SacI-KpnI fragment downstream of the ACR (construct nos. 4, 5, 8, 9, and 10) resulted in an increase of activity compared with the entire fragment, due either to potential silencer elements in these regions or to the nonspecific reduction in luciferase activity caused by increasing the size of the reporter plasmid.

Liver-enhanced transcription of the apo(a) gene is determined chiefly by the core promoter region and not the ACR. We had previously shown that the apo(a) core promoter can drive transcription in hepatic HepG2 cells, but not in kidney-derived 293 cells or HeLa cells (27). When ligated to the core promoter, the ACR is able to direct robust transcription in HepG2 cells, but produces luciferase activity barely above background in 293 kidney cells or HeLa cells. In contrast, when ligated to the SV40 early promoter, it can stimulate transcription in all three cell types (data not shown).

ACR Is Part of a LINE Element—DNA sequence analysis surprisingly revealed that the ACR is located in the 5′-untranslated region of a highly repetitive L1 element. The ACR (NheI-PvuII fragment) is located from nucleotide 386–659 relative to the transcription start site of the consensus L1 element reported by Swergold (30). This raised the question whether the enhancer activity is specific to ACR or is a universal property of all the L1s. To this end, primers L1–3 and L1–4, designed according to consensus L1 sequences, were used to generate by PCR this 274-nucleotide region from 10 random human L1 elements. When subcloned into the pGL3a expression plasmid, each of the random L1 fragments produced from 20 to 50% of the luciferase activity shown by the ACR construct in transfected HepG2 cells (data not shown). Sequence analysis of the 10 randomly selected L1 fragments revealed several scattered differences, consistent with the estimated 5% sequence differences among human L1 elements. Only two sites (Fig. 5B, sites A and B, shaded) were found to be consistently different between the ACR and the random L1s. The ACR is the only one that contains a CCGGAAG in its sequence within site A, while each of the random L1s has either CG, CA, TG, or GG at this position. At site B, the sequence of the ACR is a CAG but is GG in random L1s. (Site A, but not site B, was shown to be functionally active; see below.) Thus, the ACR may represent a
genetically different class of L1 element that can serve as a more efficient enhancer of, at least, the apo(a) gene when located in its proximity.

An Ets Element Is Critical for the Enhancer Activity of ACR—The sequence containing the site A, unique to the ACR, resembles the consensus binding site for the Ets family of transcription factors (38). Three mutations were created to test its importance in enhancing expression from the apo(a) core promoter. As shown in Fig. 3A, one, two, and five nucleotide substitutions increasingly disrupted the activity of the ACR. The effect of Ets-like proteins as trans-acting factors was demonstrated by co-transfection of either the wild type ACR(−) plasmid or the mutated mu-3 construct with 0, 1, or 3 μg of a plasmid expressing the Ets-1 protein driven by the cytomegalovirus promoter. Results are the mean of three transfections with the standard deviation. Panel C, gel shift assay was performed by incubating HepG2 nuclear extracts with a radiolabeled oligonucleotide containing wild type site A (corresponding to nucleotides 474–496 in Fig. 5A) as a probe. Lane 1 has probe only, while lanes 2–7 contain 2 μg of HepG2 nuclear extract. Various cold competitor oligonucleotides spanning this region at 100-fold excess were added in lanes 3–7. They are oligonucleotides containing: lane 3, wild type site A; lane 4, mu-1; lane 5, mu-2; lane 6, mu-3; lane 7, Sp1 oligo.

Further confirmation of the importance of this site was provided by gel mobility shift assays. An oligonucleotide encompassing site A was used as a probe to incubate with nuclear extracts prepared from HepG2 cells (Fig. 3C). A specific DNase I footprint analysis of the ACR was carried out to identify other DNA binding sites that may contribute to the ACR enhancer activity. Nuclear extracts prepared from HepG2 cells protected eight regions of the 274-nucleotide ACR region from digestion with DNase I (Figs. 4 and 5). Computer analysis of the ACR sequence revealed similarity to the consensus binding sites of transcription factors in most of the protected regions: AP-4 (footprint I); Sp1 (footprints II, V, and VI); CRE-BP (III); HNF-3β (III, VII), Ets-1 (IV); and Oct-1 (VIII). Footprint IV co-localizes with site A, the previously identified putative Ets binding site. A hallmark of Ets binding to the major groove of DNA is a DNase I protection region in the sense strand and a hypersensitive site on the antisense strand, similar to the
FIG. 4. Footprint analysis of the ACR. Anti- sense (lanes 1–3) and sense strand (lanes 4–6) of ACR were incubated with HepG2 nuclear extracts and DNase I footprint analysis was carried out. Footprint competition was also performed on the sense strand (lanes 7–9) in the presence (lane 9) or absence (lane 8) of 100-fold unlabeled Sp1 oligonucleotide (corresponding to nucleotides 429–445 of the ACR in Fig. 5A, containing an Sp1 consensus sequence) to identify Sp1-related protein binding sites on ACR. No nuclear extract protein was added to the reaction in lanes 1, 4, and 7, while lanes 2, 5, 8, and 9 contained 5 μg, and lanes 3 and 6 contained 10 μg of HepG2 nuclear extract, respectively. DNase I protected footprints are indicated by boxes and Roman numerals. Nucleotides are numbered from base 1 of the L1 sequence of Swergold (30).

The specificity of footprints II, V, and VI was demonstrated by a competition assay. When 100-fold unlabeled oligonucleotide that spans the footprint II region (named Sp1 oligo, since it matches the Sp1 consensus binding site, see Refs. 39 and 40) was incubated together with the ACR probe (sense strand) and nuclear extracts from HepG2 cells, footprints II, V, and VI were abolished, confirming the specific occupancy of these sites by Sp1-related proteins in HepG2 nuclei (Fig. 4, lanes 8 and 9). Thus, in addition to the Ets protein, transcription factor Sp1 is implicated in binding to the ACR. The identity of other binding proteins in this region awaits confirmation.

Mutagenesis of DNase I Protected Sites in the ACR—To assess the effects of the other footprint binding sites within the ACR, we subjected the entire ACR to further deletion and mutagenesis analysis. Mutations were created in these sites and tested in transfection assays in HepG2 cells. Fig. 5A shows each of the DNase I-protected sites boxed and numbered from I to VIII with mutations shown underneath in lowercase letters for base substitutions or as a line denoting a deletion mutation. As shown in Fig. 5B, mutations of footprints II, III, and VI, as well as deletion of the first 50 nucleotides, which eliminates all of footprint I and most of footprint II, do not cause a significant reduction in luciferase expression, compared with those of the wild type ACR construct. Deletion of the region including footprints VII and VIII also had no significant effect (data not shown). (It is noteworthy that the mutation in footprint VI alters site B, which was earlier noted to be one of the two sites

FIG. 5. Additional transcription factor binding sites are also involved in the ACR regulation. Panel A, sequence of ACR starts at 386 and ends at 659 relative to the start site of L1 (30). Boxes indicate the footprints as labeled in Fig. 4. Shown in boldface type are the nucleotide pairs at sites A and B that have consistent differences between the sequence of ACR and the 10 random genomic L1s that were shown to have reduced enhancer activity. In vitro mutations are indicated by the lowercase letters beneath the wild type sequence, or by a line indicating the deletion mutation. Numbers 1–3 indicate three different mutations that were tested within footprint III. Panel B, relative luciferase activity as described above is given for the wild type ACR fragment and the mutations indicated in panel A, where the sequence of the ACR differed from the 10 random L1 examples.) In contrast, destruction of the binding sites for footprints III-1, III-3, IV, and V significantly reduce the enhancer activity. The identity of the transcription factors which bind to footprint III-1 and III-3 are unknown. Footprint IV represents the target recognition site for a member of the Ets family, while footprint V is a likely target of an Sp1-related protein.

DISCUSSION

We report the screening of the 40 kb that lie between the 5’ ends of the human apo(a) and plasminogen genes for cis elements other than the apo(a) core promoter that regulate the expression of the apo(a) gene in an in vitro system. We have discovered an apo(a) transcriptional control region (ACR), located midway between the apo(a) and the plasminogen genes, that is able to confer a greater than 10-fold activation when linked in either orientation to the apo(a) minimal promoter. Sequence analysis of the ACR revealed it to be part of a LINE repeat element, whose members number up to 100,000 in the human genome. Functional analysis of 10 randomly selected, corresponding regions of other human L1 elements show that they are less efficient than the ACR in enhancing the activity of the apo(a) core promoter, and that they contain sequence differences in a site corresponding to the consensus target of the Ets family of transcription factors. We also inspected 60 additional L1 sequences available in GenBank™, and found that
they differ from the ACR in the Ets target sequence. Mutagenesis showed this Ets binding site to be indispensable for the apo(a) enhancer activity. Co-transfection of an Ets-1 expression plasmid caused a dose-dependent activation of ACR-driven expression, further implicating the role of these trans-acting proteins. DNase I footprint analysis and further site-directed mutagenesis indicate the involvement of additional factors including Sp1 in the ACR transcription enhancer activity and suggest likely interactions between several DNA binding proteins in the ACR.

Previous data from our laboratory have shown that the apo(a) minimal promoter is mediated by a liver-enriched transcription factor HNF-1α and can confer liver specific expression (27). In many cases, both promoters and more distant enhancer elements control the tissue specific expression of genes. In the case of apo(a), the ACR does not appear to be liver-specific, suggesting that the apo(a) proximal promoter is sufficient to determine tissue specificity of its expression. (Liver represents the sole organ of significant apo(a) production; much reduced levels of its mRNA have also been detected in brain and in testes of rhesus monkeys (41), but upon liver transplant, human subjects entirely convert their isofrom of circulating apo(a) from recipient to donor phenotype (42.) The ACR may function only by increasing the expression level of the rather weak, but tissue-specific apo(a) promoter. One example of an interplay between liver-specific and nonspecific elements of an opposite type is found in the apolipoprotein E gene, where tissue specificity is determined by a hepatic control region that is located 19 kb from the start of the apoE gene (43). A number of other genes are now known to be controlled by remote transcription elements located thousands of nucleotides from transcription start sites (43–46). The function of the ACR must ultimately be evaluated in its normal genomic context.

The function of the ACR must ultimately be determined by the roles of additional factors. The ACR is not a simple fixed element; its function is modulated by various factors, such as transcription factors, RNA binding proteins, and post-translational modifications like methylation. This interplay is likely to be complex and context-dependent, which is consistent with the diversity of apo(a) protein levels observed in different tissues and conditions.

Roughly 10% of these represent full-length, 6-kb elements, while only an estimated 30–60 are active elements that are capable of transposition via reverse transcription of an RNA intermediate (50). The LINE sequence within the apo(a) locus appears to be full-length. However, it does not meet the criteria of a mobile L1 element, as it contains numerous stop codons in both consensus open reading frames necessary for such activity. Nor is it a member of the Ta subclass of L1 elements to which most of the mobile elements belong (50, 51). Although it is unable to code for active mobility proteins, our data indicate that this particular L1 element is capable of interacting with DNA binding proteins and influencing the expression of an adjacent gene.

It has been proposed that methylation of CpG dinucleotides in the 5′ region of L1 sequences interferes with their expression (52). Methylation at such sites may interfere with the interaction of DNA-binding proteins such as Ets (53). Southern blotting with methylation-sensitive and insensitive restriction enzymes and a probe 5′ to the apo(a) gene L1 element indicate that the CpG dinucleotide in the Ets binding site (site A) is unmethylated in human liver cells (data not shown). This is consistent with an in vivo interaction of an Ets family protein with this site.

There have been several reported cases in which de novo insertions of L1 sequences in either the germline or somatic cells have interrupted genes and caused human disease (54–58). To our knowledge, we describe the first example of a LINE element that acts as a cellular enhancer to stimulate the expression of a nearby gene. In a related example, a subclass of Alu DNA repeats has been found to function as an estrogen receptor-dependent enhancer (59). This raises the possibility that L1 elements may serve as potentially mobile gene control elements that could influence the evolution of gene expression in mammals, as well as help to control the expression of the potentially atherogenic apo(a) gene.

REFERENCES

1. Bostom, A. G., Cupples, L. A., Jenner, J. L., Ordovas, J. M., Semaan, L. L., and Wilson, P. W. F. (1996) J. A. M. A. 276, 544–548
2. Assmann, G., Schulte, H., and von Eckardstein, A. (1996) Am. J. Cardiol. 77, 1179–1184
3. Scana, A. M., and Fless, G. M. (1990) J. Clin. Invest. 85, 1709–1715
4. Utermann, G. (1989) Science 246, 904–910
5. McLean, J. W., Tomlinson, J. E., Kaang, W. J., Eaton, D. L., Chen, E. Y., Fless, G. M., Scana, A. M., and Law, R. M. (1987) Nature 330, 132–137
6. Lackner, C., Cohen, J. C., and Hobbs, H. H. (1995) Hum. Mol. Genet. 2, 933–940
7. Harper, P. C., Gordon, B. R., and Parker, T. S. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 3847–3851
8. Miles, L. A., Fless, G. M., Levin, E. G., Scana, A. M., and Plow, E. F. (1989) Nature 339, 301–303
9. Hajjar, K. A., Garvish, D., Breslow, J. L., and Nachman, R. L. (1989) Nature 339, 303–305
10. Grainger, D. J., Kemp, P. R., Liu, A. C., Law, R. M., and Metcalf, J. C. (1994) Nature 370, 460–462
11. Law, R. M., Pearse, A. D., Kunz, L. L., Rubin, E. M., Reckless, J., Metcalf, J. C., and Grainger, D. J. (1996) J. Biol. Chem. 271, 31367–31371
12. Law, R. M., Wade, D. P., Hammer, R. E., Chiesa, G., Verstuyft, J. G., and Rubin, E. M. (1992) Nature 360, 675–677
13. Palabrica, T. M., Liu, A. C., Aronovitz, M. J., Furie, B., Lawn, R. M., and Furie, J. I. (1996) J. Clin. Invest. 98, 2921–2927
14. McQueen, E., Leffert, C. C., Liu, A. C., Chiesa, G., and Hobbs, H. H. (1992) J. Clin. Invest. 90, 50–60
15. DeMeester, C. A., Bu, X., Puppione, D., Gray, R. M., Luiss, A. J., and Rotter, J. I. (1995) Hum. Genet. 96, 287–293
16. Kraft, H. G., Kochl, S., Menzel, H. J., Sandholzer, C., and Utermann, G. (1992) Hum. Genet. 90, 220–230
17. Utermann, G., Menzel, H., Kraft, H., Duba, H., Kemmler, G., and Seitz, C. (1997) J. Clin. Invest. 80, 458–465
18. White, A. L., Hisson, J. E., Rainwater, D. L., and Lawford, R. E. (1994) J. Biol. Chem. 269, 9060–9066
19. Cohen, J. C., Chiesa, G., and Hobbs, H. H. (1993) J. Clin. Invest. 91, 1630–1636
20. Perombelon, Y. F., Soutar, A. K., and Knight, B. L. (1994) J. Clin. Invest. 93, 1481–1492
21. Garvish, D., Azrolan, N., and Breslow, J. L. (1989) J. Clin. Invest. 84, 2021–2027
22. Puckey, L. H., Law, R. M., and Knight, B. L. (1997) Hum. Mol. Genet. 6, 1099–1107
