

The number of kringle IV repeats 3–10 is invariable in the human *apo(a)* gene

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Abstract

The human *apolipoprotein(a)* (*apo(a)*) gene is a member of a family of related genes including *plasminogen*, *apo(a)rg-B* and *apo(a)rg-C*, which are clustered on chromosome 6q 2,7. Apo(a) contains ten different types of plasminogen-like kringle IV repeats (K-IV 1–10) one of which (K-IV 2) varies in number resulting in a remarkable size polymorphism of the protein. Sequence analysis of human *apo(a)* alleles and indirect evidence have suggested that K-IV 1 and K-IV 3–10 are each present once in individual alleles and that the 3' *apo(a)* region encompassing kringles IV 3–10, kringle V and the protease domain is invariable.

To directly test this, we have constructed a restriction map of the *apo(a)* gene region from genomic DNA and from a yeast artificial chromosome (YAC) (K-IV 13) which contains the entire *apo(a)* gene. The presence of a 63 kb *Clal* fragment encompassing kringles IV 3–10, kringle V and the protease domain and a 46 kb *SwaI* fragment, spanning kringles IV 5–10, kringle V and the protease domain was demonstrated by PFGE/Southern blotting in 30 unrelated subjects, who represented a range of *apo(a)* size alleles containing from 11 to 49 kringles. Our analysis demonstrates that the number of kringles IV 3–10 is invariable in the human *apo(a)* gene, suggesting that the 3' domain of Apo(a) is functionally important. © 1998 Elsevier Science B.V.

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1. Introduction

Lipoprotein(a) (Lp(a)) consists of low density lipoprotein (LDL) and a large glycoprotein, called Apolipoprotein(a) (Apo(a)), which is covalently bound to Apo B-100 in LDL by a single disulphide bridge (Utermann et al., 1987; Koschinsky et al., 1993; Brunner et al., 1993; Frank et al., 1994).

The *apo(a)* and *plasminogen* (*PLG*) genes are members of a family of related genes (*rg*) including also *apo(a)rg-B* and *apo(a)rg-C*, which are clustered on chromosome 6q 2,6-2,7 (Ichinose, 1992; Magnaghi et al., 1994; Byrne et al., 1994). The *plasminogen* gene, which is oriented in opposite direction to the *apo(a)* gene, is

located 40 kb from the 5' end of the *apo(a)* gene and the *apo(a)rg-C* starts close to its 3' end [Magnaghi et al., 1994; Byrne et al., 1994].

The *apo(a)* gene (McLean et al., 1987) has one of the most polymorphic coding regions of all genes in the human genome (Lackner et al., 1991; Kraft et al., 1992). Over 30 different sized Apo(a) glycoproteins have been demonstrated by Western blotting. Isoforms range in size from 250–800 kDa (Utermann et al., 1987). This is due to the presence of a large but variable ($n=11$ to >50 copies) number of tandemly repeated domains resembling kringle (K) IV of PLG in human Apo(a). Further, the gene contains a single homologue of plasminogen K-V, a protease domain and a 5' signal peptide.

Not all K-IV copies in the *apo(a)* cDNA are identical in sequence. Rather, there exist 11 types of K-IV modules (K-IV 1, K-IV 2A, K-IV 2B, and K-IV 3–K-IV 10), which differ in sequence and/or by small deletions [McLean et al., 1987; Mancini et al., 1995]. K-IV types 1 and 3–10 each are present once in the only two, as yet, cloned *apo(a)* cDNA, whereas K-IV 2A and 2B occur in multiple identical copies. The number of K-IV 2 repeats is represented by the size of *apo(a)* *HpaI* or

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Abbreviations: *apo(a)*, apolipoprotein(a) gene; Apo(a), Apolipoprotein(a); *apo(a)rg-B* and *apo(a)rg-C*, apolipoprotein(a) related genes A and B; ApoB-100, Apolipoprotein B-100; DIG, digoxigenin; K-IV 2, kringle IV type 2; Lp(a), Lipoprotein(a); LDL, low density lipoprotein; PFGE, pulsed field gel electrophoresis; PLG, plasminogen; YAC, yeast artificial chromosome.

KpnI fragments which show extreme allelic variation in size and correlate with the molecular weight of Apo(a) isoforms in plasma (Kraft et al., 1992; Lackner et al., 1993). Thus, variation in the number of K-IV type 2 repeats explains the Apo(a) size polymorphism. Allelic variation might, however, also exist in the number of 3' *apo(a)* K-IV 3–10 repeats. Except for two studies, one of which used RT-PCR of human liver *apo(a)* mRNA (Van der Hoek et al., 1993) and the other, which was restricted to K-IV 10 (Lackner et al., 1993) no systematic study investigating allelic variation in the number of 3' K-IV 3–10 repeats has been performed. We here have directly analysed the 3' K-IV repeats of *apo(a)* from genomic DNA and found no variation among 60 individual alleles.

2. Materials and methods

2.1. Subjects

EDTA-treated blood was drawn from 30 unrelated healthy Caucasian subjects from Austria (laboratory personnel and students of the University of Innsbruck) for analysis of the *Clal* and *SwaI* fragments. All subjects were known to be heterozygous for *apo(a)* size alleles because they had previously been analysed by PFGE/Southern blotting.

2.2. DNA isolation and plug preparation

Agarose plugs containing intact genomic DNA were prepared from each individual for pulsed-field gel electrophoresis (PFGE) exactly as described (Kraft et al., 1992). The plugs were stored in 0.5 M EDTA (pH = 8.0) at 4°C.

2.3. YAC clone

A YAC-clone was obtained by screening the YAC library of the Imperial Cancer Research Fund (Molecular Analysis of Mammalian Mutation Laboratory; head A.M. Frischauf) with the *apo(a)* cDNA clone λ A41 (McLean et al., 1987). The ID number of the clone is ICRFy900C0535 and it was tested to contain 13 K-IV repeats in its *apo(a)* gene by PFGE/Southern blotting.

2.4. PFGE (pulsed field gel electrophoresis)

Before the digestion with the respective restriction enzyme(s) the plugs were washed extensively in TE (2 × 30 min). The resulting restriction fragments were size separated in the Chefmapper system (Bio-Rad, Hercules, CA) using its autoalgorithm to determine the electrophoretic parameters. Electrophoresis was per-

formed in 1% LE agarose (Seakem, Rockland, ME) in 0.5% TAE buffer (2 M Tris, 950 mM glacial acetic acid, 5 mM EDTA, pH 8.0) at 14°C. *KpnI* fragments were separated exactly as described previously (Kraft et al., 1992). To separate smaller fragments generated by *SwaI*, *BssHIII*, and *SaII* digestion, the fragment size limits were chosen as 10–55 kb. For the *KspI/BssHIII* double digestion the chosen size limits were 150–350 kb. After electrophoresis the agarose gel was stained with ethidium bromide and nicked with 60 mJ UV light in the Gene Linker (Bio-Rad). The DNA fragments were transferred to Hybond-N membrane (Amersham, Bucks, UK) by diffusion blotting (1.5 M NaCl, 0.4 M NaOH). After baking (80°C for 20 min) and UV-linking in the Gene Linker (Bio-Rad), the nylon membrane was prehybridized and hybridized with a digoxigenin (DIG) labelled probe as described in Section 2.5. Finally, the fragments were visualized by chemoluminescence using X-ray films.

Clal restriction fragments were separated using the Gene Line apparatus (Gene Line Transverse Alternating Field Electrophoresis, Beckman, Palo Alto, CA) which yielded a better resolution of these fragments. Gene Line electrophoresis was performed in 1% LE agarose (Seakem) in 1 × TAFE buffer (200 mM Tris, 10 mM EDTA (free acid), 80 mM glacial acetic acid) at 14°C. The program for TAFE electrophoresis consisted of two stages: stage 1: 30 min, 190 mA, pulse time 4 s; stage 2: 16 h, 190 mA, pulse time 6 s.

2.5. Probes

All probes were labelled with the hapten DIG using commercial available labelling kits (Boehringer-Mannheim, Germany). Hybridization was carried out at 42°C overnight and bound probe was visualized by incubation with anti-DIG-AP (alkaline phosphatase) conjugated antibodies followed by chemiluminescence with the substrate CSPD® (Boehringer-Mannheim, Germany).

K-IV 2 specific sequences were detected with a 340 bp *PstI* fragment (= 340-probe), derived from the *apo(a)* cDNA clone λ A41 (McLean et al., 1987). The probe detects the repetitive K-IV 2 repeats and hybridizes only weakly to the K-IV 3–10 domain.

The K-IV 9–10 cDNA probe (K-IV 9–10) is a 666 bp fragment amplified by PCR from the *apo(a)* cDNA clone λ A41. The primers (upstream primer: 5'ACCTGAGAAAAGCCCTGTGGT3', downstream primer: 5'GCCCTAGGCTTGGAACTGGATG 3') were derived from the published *apo(a)* cDNA sequence (position 3347–4013) (McLean et al., 1987) employing the Oligo program (Oligo 4.1 Primer Analysis Software, Medprobe, Norway). PCR conditions were: denaturation for 40 s at 95°C, annealing for 40 s at 60°C and extension for 80 s at 72°C over 40 cycles. The PCR-derived probe was also labelled with DIG after Gene

Clean (BIO 101, Vista, CA) purification. The K-IV 9–10 cDNA probe hybridizes only with the K-IV 3–10 domain of the apo(a) gene, but not with K-IV 1 or K-IV 2 and also not with the protease domain (Fig. 1).

The pcrA-probe (Byrne et al., 1994), amplified by PCR from YAC K-IV 13, represents a 483 bp fragment, which identifies the 5' end of apo(a), plasminogen and the apo(a)-rg genes.

The protease domain probe (PD-probe) is a 400 bp fragment which was obtained by digestion of the λ A41 cDNA with the restriction enzyme ApaLI (New England Biolabs, Beverly, MA). The PD-probe hybridizes to the protease domain of the apo(a) gene, plasminogen and apo(a)-rg genes (Fig. 1).

2.6. Stripping membranes for reprobing

To hybridize the same PFGE-Blot subsequently with different probes, the Nylon membrane was washed 6 times for 30 min in 0.4 M NaOH at 62°C. Blots were stored sealed in a plastic bag at 4°C to prevent drying between hybridizations.

3. Results and discussion

3.1. Restriction map

A restriction map of the apo(a) and the adjacent apo(a)-rg-C was constructed based on analysis of genomic DNA from human lymphocytes, as well as analysis of YAC ICRFy900C0535. Genomic and YAC DNA

was digested with *Clal*, *BssHIII*, *SalI* and *SwaI* and the restriction fragments were size separated by PFGE, subjected to Southern blotting and detected with the pcrA, K-IV 9–10, and PD-probes. The number of K-IV repeats in the YAC and in genomic DNA was defined by PFGE following *KpnI* digestion. The apo(a) allele, which was analysed in the human donor, encompassed 17 K-IV repeats and that of the YAC (data not shown) 13 K-IV repeats. Most restriction sites were identical in the genomic and YAC DNA. Some methylation-sensitive restriction sites (i.e. *SalI*, *BssHIII*) were detected only in the YAC DNA (Fig. 1). The *KpnI* sites, which have been used for DNA phenotyping of the apo(a) size polymorphism (Lackner et al., 1991; Kraft et al., 1992) are located in the intron which precedes K-IV 1 and in the 1.4 kb intron separating K-IV 5 and 6 (Lackner et al., 1993). Close to these *KpnI* sites we identified *SwaI* sites (one in the intron preceding K-IV 1 and one in K-IV 5), which also flank the K-IV 2 repeats. Therefore, Southern blotting of *SwaI* digested genomic DNA followed by detection with the 340 bp probe detects the K-IV 2 repeat polymorphism (Fig. 2). *SwaI* fragments were 5 kb smaller than *KpnI* fragments, but correlated perfectly in size with *KpnI* fragments in all 34 apo(a) alleles tested. Further upstream, additional *SwaI* sites were positioned in the protease domain of apo(a) as well as in the apo(a)-rg-C.

3.2. *SwaI* RFLP in apo(a)-rg-C

Digestion with *SwaI* generated also a 46 kb fragment, which hybridized with the K-IV 9–10 probe and contains

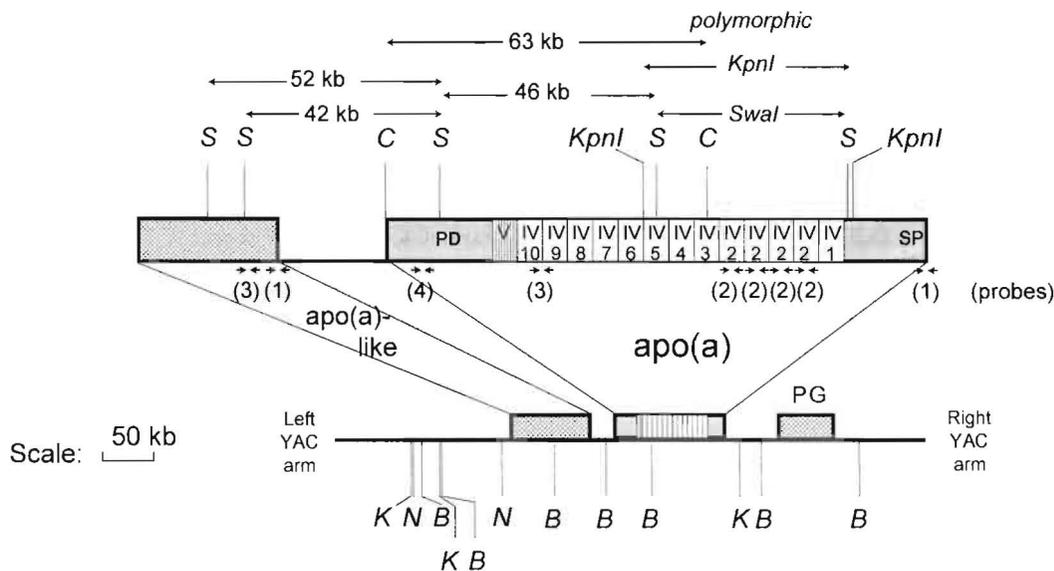


Fig. 1. Restriction map of the apo(a) gene region. The map starts with the intragenic region which separates the plasminogen gene and the apo(a) gene, followed by the apo(a) gene and the apo(a)-rg C. The analysed apo(a) allele encompasses 17 K-IV repeats. The introns are denoted by white boxes and exons by grey lines. The positions of the genomic restriction sites are indicated with arrows. The DNA probes which were used for the hybridization are indicated by small arrows: (1) pcrA-probe (Byrne et al., 1994); (2) the 340 bp probe; (3) the K-IV 9–10 probe; and (4) the PD-probe. SP, Signal peptide; PLG, plasminogen gene; PD, protease domain. Restriction enzymes: B, *BssHIII*; C, *Clal*; K, *KspI*; N, *NotI*; S, *SwaI*.

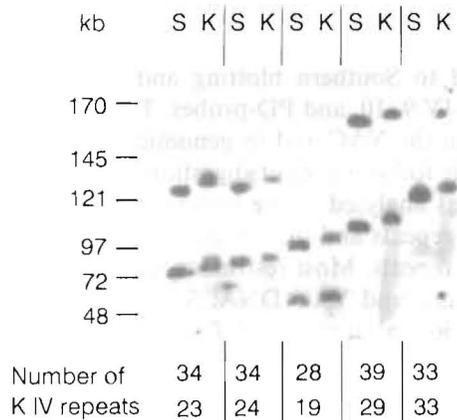


Fig. 2. Southern blot analysis of genomic DNA from five subjects. The DNA was digested with *SwaI* (S) or *KpnI* (K) and hybridized with the 340 bp probe. The *SwaI* restriction fragments correspond perfectly to the *KpnI* fragments.

K-IV 5–10, K-V and the protease domain. Genomic DNA from 17 unrelated subjects of known *KpnI* phenotype was digested with *SwaI*, subjected to PFGE/Southern blotting and hybridized with the K-IV 9–10 cDNA probe. In all individuals the 46 kb fragment was present. In addition, a polymorphic fragment was detected by the K-IV 9–10 probe (Fig. 3). Individuals had either a 42 kb, or 52 kb, or both fragments. This fragment contains the 3' end of the protease domain from the *apo(a)* gene, the signal peptide and sequences of the *apo(a)rg-C* gene.

To find out whether a RFLP or a size polymorphism in the *apo(a)rg-C* gene is the cause for the allelic 42/52 kb *SwaI* fragments, genomic DNA from an individual who was homozygous for *KpnI* and heterozygous for the *SwaI* polymorphism was double digested with *KspI/BssHII* and subjected to PFGE/Southern blotting employing the 340 probe. The double digestion generates a fragment reaching from the first *KspI* site, upstream of the *apo(a)* gene, to the *BssHII* site in the *apo(a)rg-C*. If a size polymorphism is underlying the *SwaI* polymorphism in the *apo(a)rg-C*, two *KspI/BssHII* fragments are expected in this subject. Only one was present. We conclude that the size differences of the *SwaI* restriction fragments in the *apo(a)rg-C* result from a RFLP.

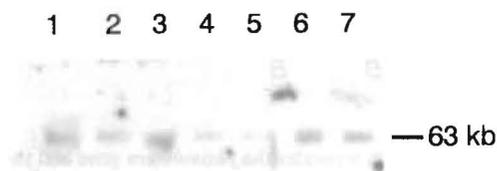


Fig. 3. PFGE/Southern blot of *ClaI* digested genomic DNA from seven unrelated individuals. The fragments were hybridized with the K-IV 9–10 probe. In all individuals a 63 kb fragment is present.

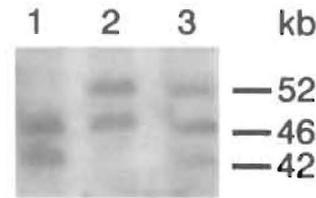


Fig. 4. PFGE Southern blot from three unrelated subjects. The genomic DNA was digested with *SwaI* and hybridized with the K-IV 9–10 probe. The 46 kb fragment is present in all investigated individuals. The individual in line 1 is homozygous for the 42 kb fragment, line 2 is homozygous for the 52 kb fragment, line 3 is heterozygous for the RFLP showing both a 42 and a 52 kb fragment.

3.3. Absence of rearrangements in the *apo(a)* 3' region

Two *ClaI* restriction sites were localized; one which was previously identified by Lackner et al. (1993) in the intron of K-IV 3. An additional *ClaI* site was identified close to the 3' end of the protease domain. Upon digestion with *ClaI* and detection with the K-IV 9–10 probe a 63 kb fragment was obtained, which encompasses K-IV 3–10, K-V and the protease domain of the *apo(a)* gene (Fig. 1).

Genomic DNA of 30 unrelated individuals was digested with *ClaI*, size separated by PFGE and the fragments were demonstrated by Southern blotting, using the K-IV 9–10 probe (Fig. 4). The 63 kb fragments were present in all investigated individuals and there was no evidence for deletions or duplications.

This result, together with the data obtained by digestion with *SwaI* provide direct evidence that no common deletions, duplications or rearrangements exist in the 3' part of the *apo(a)* gene (K-IV 3 through protease domain).

3.4. Conclusions

Our results confirm and extend previous studies of the 3' *apo(a)* gene region. In one study it was stated that a 12 kb *ClaI/KpnI* fragment which encompasses part of K-IV 3, K-IV 4 and K-IV 5 did not vary between individuals with *apo(a)* alleles of different sizes, but data and the number of analysed alleles were not provided (Lackner et al., 1993). Another study showed, by RT-PCR, that sequences corresponding to K-IV 1 and K-IV 3–10 were present in each of ten individuals studied (Van der Hoek et al., 1993). This neither excludes the possibility that one of the alleles in an individual has a deletion in the K-IV 3–10 domain of the *apo(a)* gene, nor that one or several kringles are present in more than one copy. Our study therefore is the first which provides evidence by direct analysis of 60 alleles of different sizes, that the region containing K-IV 3–10, K-V and the protease domain is invariable in size in human *apo(a)* (no deletions, duplications, or major rearrangements).

The human *apo(a)* gene therefore contains two types of K-IV domains, the highly polymorphic K-IV 2 region which has a major impact on the variation of Lp(a) plasma concentration (Lackner et al., 1991; Kraft et al., 1992) and the invariable downstream K-IV 3–10 region.

Kringles corresponding to human K-IV 3–10 were detected also in rhesus *apo(a)* cDNA (Tomlinson et al., 1989). Pesole et al. (1994) compared the sequences of human and rhesus monkey *apo(a)* genes. They found the interspecies similarity to be much higher for the K-IV 3–10 repeats than for the repetitive K-IV 2 repeats. This suggested two domains with different evolutionary dynamics.

These domains probably serve different functions. Some important functions have been localized in this region. One function of the constant domain (K-IV 3–10) is to bind to ApoB-100 in LDL. The Apo(a)/LDL interaction is initially dependent on the sequence of K-IV 6 and spacing between K-IV 6 and K-IV 9 (Frank et al., 1994). The single cysteine which is essential for formation of a stable Apo(a)/ApoB-100 complex is also within this invariable K-IV domain (K-IV 9) (Brunner et al., 1993). Lysine binding sites (LBS) have been detected in K-IV 10 (Scanu et al., 1994). and K-IV 5–8 (Ernst et al., 1995), which may be important for the antifibrinolytic activity of Apo(a) which was recently demonstrated in vivo in Apo(a) transgenic mice (Palabrica et al., 1995). No specific function of the K-IV 2 repeat domain is known. As the general function of kringle motifs is to facilitate binding of proteins to matrices, this might be also so for K-IV 2. But to which matrix and why is this domain so heterogeneous in size? The answer to the latter might be that most subjects will be heterozygous, having two *apo(a)* alleles and proteins of different size, which might be advantageous if the matrix also changes in size or shape. Recently, the evolutionary history of the *apo(a)* gene in mammals has been studied by comparing the sequences of human and hedgehog cDNA sequences (Lawn et al., 1997). Hedgehog *apo(a)* contains 31 nearly identical copies of plasminogen kringle-III and lacks a protease domain (Lawn et al., 1995). Thus the common feature of the two evolutionary different *apo(a)*s is the presence of a variable number of repetitive kringle structures, together with the company of a single cysteine which permits formation of a disulfide bond to ApoB in LDL. Whatever the function of the variable *apo(a)* domains might be, it will be intimately associated with the evolution of *apo(a)*.

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References

- Brunner, C., Kraft, H.G., Utermann, G., Müller, H.J., 1993. Cys4057 of apolipoprotein(a) is essential for lipoprotein(a) assembly. Proc. Natl. Acad. Sci. USA 90, 11643–11647.
- Byrne, C.D., Schwartz, K., Meer, K., Cheng, J.F., Lawn, R.M., 1994. The human apolipoprotein(a)/plasminogen gene cluster contains a novel homologue transcribed in liver. Arterioscler. Thromb. 14, 534–541.
- Ernst, A., Helmhold, M., Brunner, C., Petho Schramm, A., Armstrong, V.W., Muller, H.J., 1995. Identification of two functionally distinct lysine-binding sites in kringle 37 and in kringles 32–36 of human apolipoprotein(a). J. Biol. Chem. 270, 6227–6234.
- Frank, S., Durovic, S., Kostner, G.M., 1994. Structural requirements of apo-a for the lipoprotein-a assembly. Biochem. J. 304, 27–30.
- Ichinose, A., 1992. Multiple members of the plasminogen-apolipoprotein(a) gene family associated with thrombosis. Biochemistry 31, 3113–3118.
- Koschinsky, M.L., Cote, G.P., Gabel, B., Vanderhoek, Y.Y., 1993. Identification of the cysteine residue in apolipoprotein(a) that mediates extracellular coupling with Apolipoprotein B-100. J. Biol. Chem. 268, 19819–19825.
- Kraft, H.G., Köchl, S., Menzel, H.J., Sandholzer, C., Utermann, G., 1992. The apolipoprotein(a) gene-A transcribed hypervariable locus controlling plasma lipoprotein(a) concentration. Hum. Genet. 90, 220–230.
- Lackner, C., Boerwinkle, E., Leffert, C.C., Rahming, T., Hobbs, H.H., 1991. Molecular basis of apolipoprotein (a) isoform size heterogeneity as revealed by pulsed-field gel electrophoresis. J. Clin. Invest. 87, 2153–2161.
- Lackner, C., Cohen, J.C., Hobbs, H.H., 1993. Molecular definition of the extreme size polymorphism in Apolipoprotein(a). Hum. Mol. Genet. 2, 933–940.
- Lawn, R.M., Boonmark, N.W., Schwartz, K., Lindahl, G.E., Wade, D.P., Byrne, C.D., Fong, K.J., Meer, K., Patthy, L., 1995. The recurring evolution of lipoprotein(a). Insights from cloning of hedgehog apolipoprotein(a). J. Biol. Chem. 270, 24004–24009.
- Lawn, R.M., Patthy, L., Pesole, G., Saccone, C., 1997. Apolipoprotein(a): a puzzling evolutionary story. J. Mol. Evol. 44, 234–236.
- Magnaghi, P., Citterio, E., Malgaretti, N., Acquati, F., Ottolenghi, S., Taramelli, R., 1994. Molecular characterisation of the human apo(A)-plasminogen gene family clustered on the telomeric region of chromosome 6 (6Q26-27). Hum. Mol. Genet. 3, 437–442.
- Mancini, F.P., Mooser, V., Guerra, R., Hobbs, H.H., 1995. Sequence microheterogeneity in apolipoprotein(a) gene repeats and the relationship to plasma Lp(a) levels. Hum. Mol. Genet. 4, 1535–1542.
- McLean, J.W., Tomlinson, J.E., Kuang, W., Eaton, D.L., Chen, E.Y., Fless, G.M., Scanu, A.M., Lawn, R.M., 1987. cDNA sequence of human apolipoprotein (a) is homologous to plasminogen. Nature 300, 132–137.
- Palabrica, T.M., Liu, A.C., Aronovitz, M.J., Furie, B., Lawn, R.M., Furie, B.C., 1995. Antifibrinolytic activity of apolipoprotein(a) in vivo: human apolipoprotein(a) transgenic mice are resistant to tissue plasminogen activator-mediated thrombolysis. Nat. Med. 1, 256–259.
- Pesole, G., Gerardi, A., di Jeso, F., Saccone, C., 1994. The peculiar evolution of apolipoprotein(a) in human and rhesus macaque. Genetics 136, 255–260.
- Scanu, A.M., Pfaffinger, D., Lee, J.C., Hinman, J., 1994. A single point

- mutation (Trp72→Arg) in human apo(a) kringle 4-37 associated with a lysine binding defect in Lp(a). *Biochim. Biophys. Acta Mol. Basis Dis.* 1227, 41–45.
- Tomlinson, J.E., McLean, J.W., Lawn, R.M., 1989. Rhesus monkey apolipoprotein(a). Sequence, evolution, and sites of synthesis. *J. Biol. Chem.* 264, 5957–5965.
- Utermann, G., Menzel, H.J., Kraft, H.G., Duba, C.H., Kemmler, H.G., Seitz, C., 1987. Lp (a) glycoprotein phenotype. Inheritance and relation to Lp(a)-lipoprotein concentration in plasma. *J. Clin. Invest.* 80, 458–465.
- Van der Hoek, Y.Y., Wittekoek, M.E., Beisiegel, U., Kastelein, J.J.P., Koschinsky, M.L., 1993. The Apolipoprotein(a) kringle-IV repeats which differ from the major repeat kringle are present in variably-sized isoforms. *Hum. Mol. Genet.* 2, 361–366.