Annals of the New York Deademy of Science 1948, 301-12, 1995 Genetic Architecture of the Atherogenic Lipoprotein(a)

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INTRODUCTION

Lipoprotein(a) [Lp(a)] is a quantitative genetic trait in human plasma that is almost unaffected by age, diet, or life-style. The distribution of Lp(a) plasma concentrations is skewed towards lower levels in Caucasian and Asian populations and over 1000-fold differences in Lp(a) concentrations exist among individuals.¹

Extended epidemiological studies have demonstrated that high Lp(a) concentrations are associated with premature coronary heart disease (CHD) and stroke.² It is therefore important to understand the genetic mechanisms that determine an individual's Lp(a) plasma concentration.

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Lp(a) is assembled from a low-density lipoprotein (LDL) containing apolipoprotein B-100 and all the lipids and from a high molecular weight glycoprotein called apolipoprotein (a) [apo(a), FIGURE 1]. A single disulfide bridge is required to stabilize the complex.^{1,3} The gene for human apo(a) has been localized on chromosome 6q2.6–2.7 in close proximity to the gene for plasminogen (PLG) from which it evolved during primate evolution by duplications, deletions, gene conversion, and mutation.⁴ As a result, there is a high degree of homology between apo(a) and PLG. Apo(a) contains a 5' signal peptide with 100% sequence identity with the PLG signal sequence and a protease domain at the 3' end. The protease domain has no plasminlike activity but may be proteolytically active.⁵ Further, apo(a) contains two types of so-called kringles (K), one of which is homologous to KIV and the other to KV from PLG. The unique feature of apo(a) is the presence of multiple nearly identical kringle IV repeats in the apo(a) gene and protein.^{4,6} In the first sequenced apo(a) cDNA, two KIV motifs (KIV A and KIV B which differ only by silent base substitutions) occurred in multiple copies (KIV 2-29), whereas others showed small deletions (KIV-1) or minor sequence variation (KIV 30-37).

The physiological function of Lp(a) is presently unknown. The structural homology to plasminogen has suggested that Lp(a)/apo(a) may also share functional properties with PLG. A number of *in vitro* studies support this idea, e.g., apo(a) competes with PLG for binding to the endothelial PLG receptor and binds with high affinity to plasmin-degraded fibrin.⁷ These properties have also suggested mechanisms by which Lp(a) acts in a thrombogenic and/or atherogenic manner.^{2.8}

GENETIC ARCHITECTURE OF LP(a)

Apo(a) Size Polymorphism

Apo(a) exhibits a genetic size polymorphism that has been demonstrated at the protein,⁹ mRNA,¹⁰ and DNA levels.^{11,12} This polymorphism of apo(a) was originally

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detected by SDS-polyaerylamide gel electrophoresis.⁹ The apparent MW of apo(a) does range from 300 kDa to > 800 kDa, and six isoforms were initially distinguished. Improved separation techniques allowed the separation of an increasing number of apo(a) polymorphs. The most powerful method to separate apo(a) protein isoforms is SDS-agarose gel electrophoresis which has allowed identification of more than 30 apo(a) size isoforms¹³ (FIGURE 2).

The apo(a) cDNA structure with its multiple KIV repeats suggested that the size variation in the apo(a) protein is a result of a variable number of KIV repeats at the apo(a) gene locus. Pulsed-field gel electrophoresis of genomic DNA from subjects with different apo(a) protein isoforms that had been digested with appropriate restriction enzymes provided direct evidence for this assumption.^{11,12} Using enzymes (e.g., KpnI or SwaI) that do not cleave in the KIV repeat domain of apo(a), large



FIGURE 1. Model of lipoprotein(a) (adapted from Reference 1 by permission).

apo(a) fragments are obtained that differ in size by increments of 5.6 kb (FIGURE 3). This corresponds to one genomic kringle IV A/B unit.^{11,12} The DNA typing of the apo(a) size polymorphism is in analogy to the typing of other repeats (e.g., VNTRs, CA repeats, trinucleotid repeats) in the human genome except that PFGE is needed for separation of the large DNA fragments, which range in size from ~ 37 kb to > 200 kb when KpnI or SwaI are used as restriction enzymes.^{11,12} A total of 34 apo(a) DNA alleles have been identified to date, and this number will likely still increase. It has been calculated that the number of KIV repeats in individual alleles differs from 11 to more than 50.^{12,14} It has been suggested that the tremendous size variation in the apo(a) gene is due to a variable number of identical 5' KIV A/B repeats rather than to variation in the number of the "unique" 3' kringles (KIV 30–37 in the



FIGURE 2. SDS-agarose gel electrophoresis of plasma followed by immunoblotting with the monoclonal anti apo(a) antibody 1A2. Correspondence to the originally described apo(a) isoforms⁹ is given at the left side. Note the different intensities of apo(a) isoforms from some heterozygotes on the blot.

nomenclature of McLean *et al.*),⁴ but evidence for variation in the 3' kringles has also been obtained (G. Utermann, unpublished observations). A perfect correlation between the size of apo(a) DNA fragments and the size of protein isoforms has been demonstrated,¹² and apo(a) mRNA size also correlates with apo(a) isoform MW.¹⁰ Further KpnI fragments and protein isoforms cosegregate in families.^{12,15}

However some apo(a) alleles are not or only barely expressed at the protein level, and the expression of individual alleles in terms of plasma apo(a) concentration



FIGURE 3. PFGE of KpnI digested genomic DNA followed by southern blotting and hybridization with an apo(a) specific cDNA probe. Samples are arranged to create a step ladder. One step in the ladder corresponds to 5.6 kb. Differences in signal intensity in heterozygotes reflect differences in KIV repeat number (from Reference 12 by permission).

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varies widely (see below). DNA typing results in heterozygosity in 95% of Caucasians. Protein typing identifies 72% as heterozygotes and does not allow an unambiguous classification into homo- or heterozygotes in several subjects. The best information is presently obtained by performing apo(a) DNA and protein typing in parallel because information on both the size and the degree of expression of an allele is obtained. The apo(a) gene locus therefore is best described as a transcribed and translated VNTR locus that determines the excessive variation in size and concentration of the apo(a) protein.

Apo(a) Size Polymorphism and Lp(a) Concentrations

Lp(a) concentrations vary widely in the population but are amazingly stable in healthy individuals. Early family studies and twin studies have suggested that Lp(a) plasma levels are under almost complete genetic control. Heritability for Lp(a) concentrations was estimated as ≥ 0.95 in all twin studies.¹⁶ Studies of the apo(a) protein, mRNA, and DNA size polymorphism have demonstrated that the number of KIV repeats in the gene and the resulting size of the protein are inversely correlated with Lp(a) levels in plasma in all populations studied so far.^{9,11,12,15,17} The strength of this correlation is however not the same in all populations and groups.¹⁷ Both mean Lp(a) concentrations and apo(a) isoform frequencies vary significantly between populations.

Quantitative analysis revealed that the protein size polymorphism explains from 25% to 70% of the variability in Lp(a) concentrations within populations.¹⁷ Using apo(a) DNA types, from 46% to 69% of the variability in Lp(a) level in Caucasians could be attributed to the apo(a) size polymorphism.^{12,15} Together with the data from the twin studies this suggests that the apo(a) size polymorphism explains a fraction but not all of the genetic variation in apo(a) levels. Two possibilities existed: either the residual (or in some populations the major) fraction of the variability in Lp(a) concentrations is the result of further variation at the apo(a) gene locus itself or that variability in Lp(a) levels is also caused by the modulating effect of other gene loci.

Sib-Pair Linkage Analysis

Applying the principle of the sib-pair linkage approach and using apo(a) KpnI fragments for typing, it has been possible to demonstrate that Caucasian sib-pairs that share both parental apo(a) alleles have close to identical Lp(a) concentrations (intraclass correlation 0.95), whereas in those that had no parental allele in common, there was no correlation in Lp(a) levels.^{12,15} The data from the sib-pair linkage analysis clearly show that variation at the apo(a) locus explains over 90% of the variation in Lp(a) concentrations among Caucasian individuals. The conclusion from the combined population, sib-pair linkage, family, and twin studies is that Lp(a)concentrations are under almost complete genetic control. This control is almost entirely by the apo(a) locus. The measured variation (= size polymorphism) at the apo(a) locus does however explain only about 50% of the variability in Lp(a) concentrations in Caucasians. It follows that there must be further variation at the apo(a) locus, which might be sequence variation in coding regions or in regulatory elements of the apo(a) gene. The types and quantitative effects of factors controlling Lp(a) plasma concentrations that emerged from these studies are illustrated in the pie diagram of FIGURE 4.

Sequence Polymorphism in Kringle IV-37 of the Apolipoprotein(a) Gene

In order to detect variation in the coding region of the apo(a) gene, we started to analyze the 3' "unique" kringle IV motives in apo(a). RT-PCR cloning from human liver poly A+ RNA of the apo(a) KIV 30-37 domain followed by sequencing revealed a single nucleotide exchange $(T \rightarrow C)$ in position 12605 which is in the ultimate kringle IV domain (K37) of apo(a) and results in a Met (ATG) to Thr (ACG) exchange in position 4168 of apo(a). The respective alleles were designated 4168^{Met} and 4168^{Thr} (Ch. Brunner, H. G. Kraft, H. J. Müller, G. Utermann, unpublished). This substitution has previously been observed by protein sequencing⁶ and RT-PCR analysis of liver mRNA,¹⁸ but only a few alleles were analyzed in these studies. In order to investigate whether this substitution represents a rare mutation or a polymorphism, a PCR-based restriction assay of genomic DNA was developed which demonstrated that this substitution represents a common polymorphism (FIGURE 5). In 231 unrelated Tyroleans, the frequencies for the K37^{Met} and K37^{Thr} alleles were 0.34 and 0.66 respectively.



FIGURE 4. Pie diagram illustrating the type and effects of factors controlling Lp(a) concentrations in healthy Caucasians.

All 231 subjects had either the 131 bp or 104 bp fragment or both. Thus there is no evidence for a homozygous deletion of K 37 in any of the tested individuals.

The Met/Thr polymorphism was further analyzed on 40 individual apo(a) alleles separated by PFGE from subjects, which appeared as double heterozygotes for KIV repeat number and the Met/Thr variation. The analysis of Met/Thr types on separated apo(a) alleles served a double purpose. First, we wanted to determine the phase of the Met/Thr polymorphs in relation to the apo(a) size polymorphs in heterozygotes. Second, we wanted to know whether K 37 might be duplicated or deleted in some subjects. A duplicated K 37 in a Met/Thr "heterozygote" might be recognized by the presence of the I31 bp and 104 bp fragments on one allele. Deletion of K 37 will result in the absence of both PCR products from the respective size fragment. Identification of both situations requires separation of apo(a) alleles.

Genomic DNA from Thr/Met heterozygotes was digested with Kspl. This results in fragments that contain the entire KIV repeat domain of the apo(a) gene, all unique 3' KIV kringles (30–37), kringle V, and the protease domain (S. Köchl, H. G. Kraft, G. Utermann, unpublished). Apo(a) Kspl fragments were separated by PFGE.¹⁹ Kspl fragment size corresponded to the size of Kpnl fragments previously determined for the same subjects (see Reference 19 and data not shown).



Met Met/Thr Thr

+ Kspl Digest

FIGURE 5. Demonstration of the Met/Thr polymorphism in kringle 37 at position 4168 of apo(a) by agarose gel electrophoresis. Oligonucleotide primers specific for exon 1 of kringle 37 were used to amplify a 131 bp fragment (nucleotides 12579-12709) by PCR. A deliberate mismatch in the upper primer creates a KspI site in an allele carrying the ACG (4168^{Thr}) codon. KspI digestion followed by agarose gel electrophoresis results in a 104 bp fragment in the presence of this allele and in a 131 bp fragment in the presence of the 4168^{Met} allele. Lane 1, Met/Met homozygote; lane 2, Met/Thr heterozygote; lane 3, Thr/Thr homozygote.

PCR amplification/KspI digestion of the KIV-37 target sequence from separated alleles resulted in the separation of the Thr/Met polymorphs in all 20 heterozygotes. Thus no evidence for duplication or deletion of this kringle was obtained.

In agreement with previous studies,^{14,18} this suggests that the 3' kringle domain of the apo(a) gene is invariably in contrast to the enormous size heterogeneity of the 5' KIV A/B repeats.

KpnI fragments [which reflect the apo(a) size polymorphism] were known for all subjects typed for the Thr/Met polymorphism, and in all double heterozygotes the phase of the two polymorphisms was also determined (see above). We therefore were able to test for the total sample whether an allelic association (linkage disequilibrium) exists between the Thr/Met and apo(a) size polymorphisms. An allelic association of the sequence polymorphism with the apo(a) size polymorphism (KIV repeat number) was indeed present, suggesting a recent origin of the Met/Thr polymorphism (data not shown).

Relation of Thr/Met Types to Lp(a) Plasma Concentration

Lp(a) plasma concentrations were determined by ELISA and compared between the three phenotypic classes (TABLE 1). No significant difference in mean and median Lp(a) concentration between types was noted. Though the mean Lp(a) concentration increased from 16.6 mg/dl (Met/Met) to 17.3 mg/dl (Met/Thr) and 19.1 mg/dl (Thr/Thr), the differences were not significant. Moreover they were fully explained by different frequencies of the apo(a) size alleles in the three groups. Calculation of Lp(a) concentrations expected in the three groups considering type specific means and frequencies of types resulted in values very close to the observed ones (data not shown). Thus this polymorphism does not contribute to further elucidating the genetic architecture of the quantitative Lp(a) trait. The Met/Thr polymorphism could also be demonstrated by single strand conformation polymorphism (SSCP) analysis of the same PCR amplified K 37 fragments that were used for restriction analysis with KspI. Phenotyping of the Thr/Met polymorphism in 20 subjects by both methods resulted in complete agreement.

Apo(a) Promoter Variation and Lp(a) Concentration

The signal sequence of apo(a) is separated by a 14 kb intron from the first kringle sequence in apo(a) and is identical to four other signal sequences from related genes.^{20,21} This has made identification of the apo(a) promoter difficult. Recently the apo(a) promoter was identified, cloned, and sequenced.^{19–21} Differences in promoter sequence and activity were reported between two subjects with different Lp(a) concentrations, and it was concluded that sequence variation in the apo(a) promoter causes differences in apo(a) promoter activity and Lp(a) concentrations.²² One of the differences identified was the number of pentanucleotide repeats in individual promoters.

In order to see whether apo(a) promoter variation indeed may effect Lp(a) levels, we have determined the numbers, frequencies, and effects on Lp(a) levels of a pentanucleotide repeat polymorphism in the apo(a) promoter. Alleles with 7 to 11 repeats were detected in 85 unrelated Tyroleans with those with 8 and 9 repeats being most frequent (TABLE 2). No effect of this polymorphism on Lp(a) levels was detected when apo(a) KpnI alleles were not considered in the analysis. Considering apo(a) allele size may suggest that the pentanucleotide repeat polymorphism effects Lp(a) levels (TABLE 2), but a careful statistical analysis and possibly analysis of a larger sample will be required to clarify this.

TABLE 1. Apo(a) Kringle 37 Met/Thr Polymorphism: Phenotype Frequencies and Phenotype Associated Lp(a) Plasma Concentration in a Tyrolean Population $(n = 231)^{\mu}$

Phenotype	n (%)	Lp(a) (mg/dl)
Met/Met	9	16.6
Met/Thr	51	17.3
Thr/Thr	40	19.1

 $^{\prime\prime}\chi^{2} = 3.497 \ (p = 0.174).$

Phenotype	п	Lp(a) (mg/dl)	∠Lp(a) ⁴ (mg/dl)
7/8	1	1.3	- 28.5
8/8	42	22.0	5.9
8/9	19	14.5	0.2
8/10	9	28.0	-1.4
8/11	3	11.6	-6.3
9/9	6	23.1	-().5
9/10	3	5.0	-9.7
10/10	2	5.6	-23.7
Σ	85	24.1	1.3

TABLE 2. Pentanucleotide Polymorphism in the Apo(a) Promoter: Phenotype Frequencies and Associated Lp(a) Concentrations

"Lp(a) concentration difference between Kpn type specific concentration (determined in a normal Tyrolean population; H. G. Kraft, F. Kronenberg and G. Utermann unpublished) and measured plasma concentration. A negative value means that the measured Lp(a) concentration is lower than expected according to the apo(a) size polymorphism.

APOLIPOPROTEIN(a) POLYMORPHISM AND CORONARY HEART DISEASE

Numerous case-control studies have demonstrated an association of elevated Lp(a) plasma concentrations with premature coronary atherosclerosis and myocardial infarction (reviewed in Reference 2). More recent nested prospective studies were controversial,²³⁻²⁵ and the Helsinki Heart Study has cast doubt on the notion that Lp(a) concentrations are a primary risk factor for myocardial infarction. Lp(a)levels may not be under such strict genetic control in all groups (e.g., patients with atherosclerotic vascular disease) as has been demonstrated for healthy population samples (see above). The associations observed in case-control and even in relatively short-time prospective studies may therefore be biased by an effect of atherosclerotic vascular disease on Lp(a) levels. In order to circumvent this problem, we have started to analyze the apo(a) size polymorphism in patients with coronary heart disease. A study of the apo(a) protein polymorphism in CHD patients and controls from six ethnic groups around the world which included over 1000 CHD patients demonstrated that low molecular apo(a) isoforms [which are associated with high Lp(a) levels in the general population] are significantly overrepresented in the CHD patients compared to controls.^{26,27} This suggested that variation at the apo(a) gene locus that is not affected by disease and stable throughout life is associated with

<i>Kpn</i> I Allele Number	CHD Patients $(n = 248)$	Controls $(n = 448)$	
1-6	0	1.2	
7_9	6.0	2.2	
10-12	16.1	11.8	
13-15	12.9	13.8	
16-32	65.0	71.0	
	0510		

TABLE 3. Frequencies (%) of Binned Apo(a) KpnI Types in Patients with Angiographically Defined Coronary Heart Disease and Controls⁴

"Binning was performed as outlined in Reference 12. $\chi^2 = 12.45$ (d.f. = 4, p < 0.025).

CHD.^{26,27} We now have extended these studies and have demonstrated that apo(a) DNA variation [i.e., the apo(a) KpnI polymorphism] is also associated with CHD. Analysis by pulsed field gel electrophoresis of KpnI fragments from 124 patients with angiographically defined coronary heart disease and from 224 healthy controls demonstrated that small KpnI fragments are significantly more frequent in CHD patients than in controls (p < 0.025) (TABLE 3). The small, low KIV copy number apo(a) KpnI fragments are those that are associated with high Lp(a) concentrations in the general population^{11,12} but also in CHD patients (data not shown). We consider the demonstration that DNA variation at the apo(a) gene locus that determines Lp(a) concentration is also associated with CHD as evidence that apo(a)/Lp(a) is causally related to CHD.

CONCLUSION

Lp(a) concentrations in healthy Caucasian subjects are largely determined by variation at the apo(a) gene locus on chromosome 6q2.6-2.7. This variation has been dissected into two components: (1) variation in the number of plasminogen-like kringle IV repeats in the apo(a) gene explains roughly 50% of the within population variability in Lp(a) levels; (2) the residual genetic variability is explained by unrecognized sequence variation in the apo(a) gene. Neither a Met/Thr polymorphism in KIV-37 (codon 4168) nor a pentanucleotide polymorphism in the apo(a) promoter seems responsible for a major fraction of Lp(a) level variability. Sequence variation in apo(a) that relates to Lp(a) levels still needs to be identified. Lp(a) concentrations are associated with premature coronary heart disease. It is however still controversial whether Lp(a) is a primary risk factor for CHD and to which extent Lp(a) level variation in CHD patients is controlled by the apo(a) locus. We have related the number of KIV repeats in the apo(a) gene to the occurrence of CHD and found a significant association of low KIV copy number alleles with CHD. This demonstrates that apo(a) gene variation relates to both Lp(a) concentration and CHD, suggesting that the association of Lp(a) with CHD is indeed causal.

REFERENCES

- 1. UTERMANN, G. 1989. The mysteries of lipoprotein(a). Science 246: 904-910.
- SCANU, A. M. & G. M. FLESS. 1990. Lipoprotein(a). Heterogeneity and biological relevance. J. Clin. Invest. 85: 1709–1715.
- BRUNNER, C., H. G. KRAFT, G. UTERMANN & H. J. MÜLLER. 1993. Cys4057 of apolipoprotein(a) is essential for lipoprotein(a) assembly. Proc. Natl. Acad. Sci. USA 90: 11643– 11647.
- MCLEAN, J. W., J. E. TOMLINSON, W-J. KUANG, D. L. EATON, E. Y. CHEN, G. M. FLESS, A. M. SCANU & R. M. LAWN. 1987. cDNA sequence of human apolipoprotein(a) is homolgous to plasminogen. Nature 300: 132-137.
- SALONEN, E. M., M. JAUHIAINEN, L. ZARDI, A. VAHERI & C. EHNHOLM. 1989. Lipoprotein(a) binds to fibronectin and has a serine proteinase activity capable of cleaving it. EMBO J. 8: 4035–4040.
- KRATZIN, H., V. W. ARMSTRONG, M. NIEHAUS, N. HILSCHMAN & D. SEIDEL. 1987. Structural relationship of an apolipoprotein(a) phenotype (570 kDa) to plasminogen: homologous kringle domains are linked by carbohydrate-rich regions. Biol. Chem. Hoppe-Seyler 368: 1533-1544.
- LOSCALZO, J., M. WEINFELD, G. M. FLESS & A. M. SCANU. 1990. Lipoprotein(a), fibrin binding, and plasminogen activation. Arteriosclerosis 10: 240–245.

- EZRATTY, A. & J. LOSCALZO, 1992, Lipoprotein(a): a risk factor with novel mechanisms for the development of atherothrombotic disease. Adv. Endocrinol. Metab. 3: 237–257.
- UTERMANN, G., H. J. MENZEL, H. G. KRAFT, H. C. DUBA, H. G. KEMMLER & C. SETTZ. 1987. Lp(a) glycoprotein phenotypes. Inheritance and relation to Lp(a)-lipoprotein concentrations in plasma, J. Clin. Invest. 80: 458–465.
- KOSCHINSKY, M. L., J. E. TOMLINSON, T. F. ZIONCHECK, K. SCHWARTZ, D. L. EATON & R. M. LAWN, 1991. Apolipoprotein(a): expression and characterization of a recombinant form of the protein in mammalian cells. Biochemistry 30: 5044–5051.
- LACKNER, C., E. BOERWINKLE, C. C. LEFFERT, T. RAHMIG & H. H. HOBBS, 1991. Molecular basis of apolipoprotein(a) isoform size heterogeneity as revealed by pulsedfield gel electrophoresis. J. Clin. Invest. 87: 2153-2161.
- KRAFT, H. G., S. KÖCHL, H. J. MENZEL, C. SANDHOLZER & G. UTERMANN, 1992. The apolipoprotein(a) gene: a transcribed hypervariable locus controlling plasma lipoprotein(a) concentration. Hum. Genet. 90: 220-230.
- KAMBOH, M. I., R. E. FERRELL & B. A. KOTTKE. 1991. Expressed hypervariable polymorphism of apolipoprotein(a). Am. J. Hum. Genet. 49: 1063–1074.
- LACKNER, C., J. C. COHEN & H. H. HOBBS. 1993. Molecular definition of the extreme size polymorphism in apolipoprotein(a). Hum. Mol. Genet. 2: 933–940.
- BOERWINKLE, E., C. C. LEFFERT, J. LIN, C. LACKNER, G. CHIESA & H. H. HOBBS. 1992. Apolipoprotein(a) gene accounts for greater than 90% of the variation in plasma lipoprotein(a) concentrations. J. Clin. Invest. 90: 52–60.
- AUSTIN, M. A., C. SANDHOLZER, J. V. SELBY, B. NEWMAN, R. M. KRAUSS & G. UTERMANN. 1992. Lipoprotein(a) in women twins: heritability and relationship to apolipoprotein(a) phenotypes. Am. J. Hum. Genet. 51: 829–840.
- SANDHOLZER, C., D. M. HALLMAN, N. SAHA, G. SIGURDSSON, C. LACKNER, A. CSÁSZÁR, E. BOERWINKLE & G. UTERMANN. 1991. Effects of the apolipoprotein(a) size polymorphism on the lipoprotein(a) concentration in 7 ethnic groups. Hum. Genet. 86: 607– 614.
- VAN DER HOEK, Y. Y., M. E. WITTEKOEK, U. BEISIEGEL, J. J. P. KASTELEIN & M. L. KOSCHINSKY. 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.
- KRAFT, H. G., N. MALGARETTI, S. KÖCHL, F. ACQUATI, G. UTERMANN & R. TARAMELLI. 1993. Demonstration of physical linkage between the promoter region and the polymorphic kringle IV domain in the apo(a) gene by pulsed field gel electrophoresis. Genomics 17: 260–262.
- 20. ICHINOSE, A. 1992. Multiple members of the plasminogen-apolipoprotein(a) gene family associated with thrombosis. Biochemistry 31: 3113-3118.
- MALGARETTI, N., F. ACQUATI, P. MAGNAGHI, L. BRUNO, M. PONTOGLIO, M. ROCCHI, S. SACCONE, G. DELLA VALLE, M. D'URSO, D. LEPASLIER, S. OTTOLENGHI & R. TARA-MELLI. 1992. Characterization by yeast artificial chromosome cloning of the linked apolipoprotein(a) and plasminogen genes and identification of the apolipoprotein(a) 5' flanking region. Proc. Natl. Acad. Sci. USA 89: 11584–11588.
- WADE, D. P., J. G. CLARKE, G. E. LINDAHL, A. C. LIU, B. R. ZYSOW, K. MEER, K. SCHWARTZ & R. M. LAWN. 1993. 5' control regions of the apolipoprotein(a) gene and members of the related plasminogen gene family. Proc. Natl. Acad. Sci. USA 90: 1369– 1373.
- ROSENGREN, A., L. WILHELMSEN, E. ERIKSSON, B. RISBERG & H. WEDEL. 1990. Lipoprotein(a) and coronary heart disease: a prospective case-control study in a general population sample of middle aged men. Br. Med. J. 301: 1248-1251.
- JAUHIAINEN, M., P. KOSKINEN, C. EHNHOLM, H. M. FRICK, M. MÄNTTÄRI, V. MANNINEN & J. K. HUTTUNEN. 1991. Lipoprotein(a) and coronary heart disease risk: a nested case-control study of the Helsinki Heart Study participants. Atherosclerosis 89: 59-67.
- SIGURDSSON, G., A. BALDURSDOTTIR, H. SIGVALDASON, U. AGNARSSON, G. THORGEIRS-SON & N. SIGFUSSON. 1992. Predictive value of apolipoproteins in a prospective survey of coronary artery disease in men. Am. J. Cardiol. 69(16): 1251–1254.

- SANDHOLZER, C., E. BOERWINKLE, N. SAHA, C. TONG & G. UTERMANN, 1992. Apolipoprotein(a) phenotypes, Lp(a) concentration and plasma lipid levels in relation to coronary heart disease in a chinese population: evidence for the role of the apo(a) gene in coronary heart disease. J. Clin. Invest. 89: 1040-1046.
- SANDHOLZER, C., N. SAHA, J. D. KARK, A. REES, W. JAROSS, H. DIEPUINGER, F. HOPPICHLER, E. BOERWINKLE & G. UTERMANN, 1992, Apo(a) isoforms predict risk for coronary heart disease: a study in six populations. Arterioscler. Thromb. 12: 1214–1226.

DISCUSSION OF THE PAPER

P. O. KWITEROVICH (Johns Hopkins University, Baltimore, Md.): Is it possible that heterogeneity in the carbohydrate side chains could be contributing to Lp(a) variation in the levels in plasma?

UTERMANN: NO.

KWITEROVICH: Why not?

UTERMANN: Carbohydrate chains are on almost each apo(a) kringle. Theoretically one could have an effect of carbohydrate heterogeneity on secretion, but there's no evidence for that actually. It's the copy number of kringle 4 repeats in the DNA and other mutations in the gene that determine the concentration of Lp(a) in plasma. That doesn't have to do with the carbohydrates. It is highly unlikely that there are systematic differences in carbohydrate side chains among isoforms. Mutations at glycosilation sites are possible but would affect only one of the many kringle repeats.

QUESTION: Do you have any data about different therapies or plasma half-life so. Can the bigger gene production be related to protein stability?

UTERMANN: Of course that is one of the intriguing questions. How does the number of repeats in the gene determine differences in levels. What is known is that Lp(a) plasma levels are almost entirely controlled by synthetic rate and not by degradation. That has been shown by turnover studies by Kostner and coworkers in Austria and more recently by Brewer's group at the NIH. So it must be the synthesis. What we have done in collaboration with Christoph Brunner and Joachim Müller from Boehringer-Mannheim—and that is unpublished studies—is we have transfected hep G2 cells with recombinant apo(a) that differed only in the number of kringle repeats and we have then looked at whether what we see in the population *in vivo* we can reproduce in the cell system and indeed we can.

R. W. WISSLER (University of Chicago, Chicago, Ill.): This is a very exciting field I think, and Paul Roheim from New Orleans and Dr. Kasumi from Nehan University working in our laboratory have been tracking the Lp(a) in the Ped-A lesions and in the serum of Ped-A individuals. He has demonstrated a much different localization of Lp(a) in the lesions then apo(b) for example and that there's a very strong correlation between the level in the serum and the level we find in the developing lesions in the Ped-A autopsy specimens. I wonder what the current thinking is about how Lp(a) produces the intensification of atherosclerosis that it apparently does.

UTERMANN: Your Ped-A studies obviously confirm what my former coworker Ulrike Beisiegel has published on apo(a) deposition in atherosclerotic plaques. She also observed that apo(a) in plaques relates to Lp(a) plasma levels. How Lp(a)comes to the plaque is not yet known. The data reported by Lawn's group in Stanford show that Lp(a) is taken up by macrophages and that may well be the route of entry,

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but I can't exclude that there are other routes and it's not known for the moment. May I ask you a question Dr. Wissler. You say the distribution of apo(a) in the lesion is different from that of apo B, in which cell is apo(a)?

WISSLER: Most of it is not in cells. It's true that some of it is in macrophages, if there are very many macrophages. We don't find very many in this age group between 15 and 34 years of age. But most of it is attached either on the intimal surface of the artery or it's very heavily concentrated on many of the other fiber proteins in the matrix, both collagen and elastin. We don't find it in the diffuse way that we find apo B which is frequently very diffusely distributed throughout the developing lesion and so it looks quite different in this early stage of atherosclerosis than what some other people have described as being more or less similar to apo(b).

G. BROWN (University of Washington, Seattle, Wash.): We've looked at the Lp(a) picture in our fats patients, patients who are largely selected for a high apo B and therefore have high LDL. We find in the patients who are not treated, the placebo patients in our study, that there's a very strong relationship between Lp(a) level and the amount of coronary disease. The controversy about Lp(a) relates to the question whether its thrombogenic or atherogenic.

UTERMANN: Or both.

BROWN: Or both. Our conclusions seem to be that it appears more atherogenic because it does indeed relate to disease progression in the untreated patients over the course of 2½ years. Of interest, if you lower LDL, this relationship completely disappears and disease progression in fact doesn't occur at regression. I'd like your speculations on whether it is thrombogenic or atherogenic or both.

UTERMANN: I have difficulty believing that it is thrombogenic and the reason is the following. We have seen here this beautiful picture showing the delicate balance between thrombogenic and fibrinolytic pathways. If you haven't really fine tuned this system, either you get bleeding or you get thrombosis. When you have a substancesay Lp(a) that varies a thousandfold in concentration, I would expect that at the extremes you should see very severe clinical manifestations if the substance indeed affects the balance, and you don't see that. So personally I have difficulties believing that Lp(a) or apo(a) interferes *in vivo* with thrombogenesis. There are a lot of nice *in vitro* data, but I think there is no real evidence for that. That's my personal view.

M. A. GIMBRONE (*Harvard Medical School, Boston, Mass.*): I'm concerned actually about the use of the term thrombogenic as if the only thing that thrombin does is to generate a large plug that we see clinically as a final event. There are plenty of cell biological implications for tiny bits of thrombin being generated at the interface that influence the behavior of all the cells we've been talking about at any stage in the progression that you want to discuss. So I think sometimes we are captives of the terms, the pathological terms, thrombosis. I think there are bits of the mechanisms molecularly that may be very relevant, and I think that oftentimes we don't have ways of evaluating that at different stages of progression, certainly in man, until the clinical events occur. So it's just a response that has to do with where these different pieces fit in. Indeed I think they fit in everywhere at the level of molecular mechanism.