thematic review series

Lipoprotein (a): Coming of Age at Last

Joseph L. Witztum*,[1] and Henry N. Ginsberg†

Division of Endocrinology and Metabolism,* Department of Medicine, University of California, San Diego, La Jolla, CA; and Department of Medicine,† Columbia University College of Physicians and Surgeons, New York, NY

In 1963, the geneticist Kare Berg was seeking to define lipoprotein differences between individual human sera, and through a simple but ingenious set of immunological investigations of human sera, discovered a new antigen that was associated with low density lipoproteins (LDL) (1). He shortly showed that this new antigen was a genetic trait and proposed it be called Lp(a): the Lp referring to the lipoprotein, and the small "a" in brackets as this was the accepted terminology at that time for naming antigens in human immunogenetics. Thus, as originally intended, the term Lp(a) referred to the antigenic structure of the new antigen and not the Lp(a) lipoprotein as it is used today. This terminology has subsequently led to much confusion in the literature and among clinicians because of the similarity of "apolipoprotein A" (apoA-I of HDL) with the "apolipoprotein (a)" of Lp(a). However, the use of "a" to describe the antigen protein linked to LDL predated the development of the apolipoprotein terminology by a number of years.

Berg's very first paper was remarkably prescient, as he already described family studies showing that Lp(a) behaved as a genetic trait, and this work was rapidly extended (2). Early work using relatively insensitive techniques led to the concept that people were either Lp(a)+ or Lp(a)− and as early as 1974, Berg and colleagues reported a higher frequency of the Lp(a)+ phenotype in patients with coronary heart disease compared with healthy controls (3). As techniques for measuring Lp(a) improved, the association of Lp(a) with cardiovascular disease (CVD) was more fully developed, so that by the late 1980s it was clear that Lp(a) represented a highly prevalent inherited risk factor for CVD, but unlike most classical risk factors, Lp(a) levels were relatively unresponsive to either diet, environmental variables, or available drugs (4).

The cloning and sequencing of apo(a) in 1987 by Lawn and colleagues (5, 6) revealed many surprises and kindled a new burst of interest in Lp(a). They showed that the apolipoprotein (a) gene, termed *LPA,* evolved from the plasminogen (*PLG*) gene and that it contained multiple so-called kringle (K) domains common in coagulation factors, as well as a mutated protease domain that lacked the proteolytic activity of plasmin. Their seminal work provided new insights into the complexity of the genetics of LPA, which helped to explain the widespread variation in plasma Lp(a) levels. Among the various Ks derived from the *PLG* gene, the KIV had expanded and diversified by mutation into 10 different types termed KIV1–10. One of these, the KIV-2, was found to exist in multiple copies such that few individuals have the same two alleles. Indeed more than 95% of the population has heterozygosity for the copy number variation (CNV) of KIV-2, which can vary from as few as 3 to > 40 copies, leading to different sized apo(a) proteins (7). Thus, most individuals generate two apo(a) proteins of different size. The apo(a) allele with a small CNV of KIV-2 will yield an Lp(a) with a shorter apo(a) protein, which is associated with high plasma levels, while an apo(a) allele with a high CNV will yield an Lp(a) with a longer apo(a), associated with lower plasma Lp(a) levels. Indeed, it was shown soon after in a small population that the KIV-2 CNV predicts Lp(a) levels and CVD risk (8).

Over the next few decades, extensive studies undertook to define the mechanisms by which Lp(a) promoted atherosclerosis, and in a broad sense these focused on the possibility that apo(a) might somehow interfere with the role of plasminogen in promoting fibrinolysis on the one hand, and on the other, on the possibility that its atherogenicity depended on properties associated with the LDL moiety or proatherogenic properties of apo(a) itself (9). More recently, the discovery that Lp(a) among all lipoproteins was a carrier of proinflammatory oxidized phospholipids (OxPLs) added yet another possible explanation for its atherogenicity (10, 11). Indeed, it is popular to say that Lp(a) promotes "atherothrombosis," but as with so many other aspects of Lp(a) biology, whether Lp(a) interferes with normal coagulation properties in vivo and the mechanisms by which it promotes atherosclerosis are still unresolved issues. Indeed, there are a whole host of fundamental questions regarding the biology and metabolism of Lp(a) that remain to this day unanswered, such fundamental questions as: what governs its synthesis and regulation,

J.L.W. has patents and patent disclosures for the use of oxidation-specific antibodies that are held by UCSD and is a consultant for Ionis Pharmaceutical, Intercept, Cymabay, and Prometheus. H.N.G. is a consultant for Merck, Sanofi, Regeneron, Amgen, Lilly, Boerhinger Ingelheim, AstraZeneca, and Kowa.

DOI 10.1194/jlr.E066985

[1]To whom correspondence should be addressed.
e-mail: jwitztum@ucsd.edu

how and where is apo(a) covalently linked to apoB-100 of LDL to form Lp(a), and what governs Lp(a) metabolism and clearance. There is indeed much to be learned.

Despite ongoing advances in epidemiological studies and insights from mechanistic studies, interest in Lp(a) as a CVD risk factor waned until the recent decade. Since the 1990s, Lp(a) remained for the most part a focus of academic study among a limited cohort of investigators. Even to the present time, it is not widely known as a CVD risk factor among most cardiologists, clinicians, or even endocrinologists, and even fewer understand what it is, what are its metabolic properties, what regulates its genetics, and what factors govern its levels. At the clinical level, with the exception of some dedicated lipidologists and cardiologists, physicians are not measuring Lp(a) as a cardiac risk factor in their patients, even in those at high risk or with established CVD. In part, this has stemmed from the fact that there are no standardized ways to measure and report Lp(a) levels, and cut points that represent "normal" or sufficiently elevated levels to require treatment are not well defined. For the most part, measurements are not available in routine laboratories and must be sent away to specialty referral laboratories at considerable expense. Most likely, the primary reason clinicians fail to measure Lp(a) is that there is no specific therapy to substantially lower elevated levels. Although niacin is known to be capable of lowering plasma Lp(a) levels 25–40% or even more in some patients, its use is associated with considerable side effects and has been greatly restricted due to the perception that it is unsafe and does not reduce CVD risk.

However, in recent years a remarkable resurgence of interest in Lp(a) has occurred. This has been fueled by meta-analyses of by now a large body of epidemiological studies, which have consistently shown modest associations of Lp(a) with nonfatal myocardial infarction and coronary death (12, 13). Moreover, a series of genome-wide association studies (GWASs) and Mendelian randomization studies, where confounding variables that limit epidemiological studies are minimized, show a linear and potent relationship to CVD risk with odds ratios for the highest Lp(a) values that are 3-fold above the lowest reference Lp(a) values (14–16). In such studies, as for example, reflected by single nucleotide polymorphisms that are associated with life-long elevated plasma Lp(a) levels, elevated Lp(a) appears to be a key driver of CVD. Indeed, these modern, high-throughput assays applied to exceedingly large populations have provided high confidence in observations of the strong relationship of Lp(a) to CVD made much earlier in smaller populations, and to borrow a phrase used by Sekar Kathiresan, are "more 'redux' than new" (17), reflecting the strong scholarship of the earlier investigators. Moreover, recent GWASs have revealed that the *LPA* gene is also the single most powerful genetic risk factor for calcific aortic valve stenosis (CAVS), with ever-new studies confirming this association (18–20). Based on the emerging epidemiological, GWAS, and Mendelian randomization data supporting a causal relationship of Lp(a) levels to CVD and CAVS, a European consensus panel has proposed that Lp(a) values above ~50 mg/dl (or 125 nmol/L) pose a major risk (21). Based on preliminary prevalence data, adoption of this cutoff indicates that up to 20% of populations in the United States and Europe are at risk. However, Lp(a) levels vary considerably in different ethnic populations and much effort will be required to both standardize measurements and develop ethnic appropriate cutoff values.

As noted above, even the recognition that Lp(a) is a major risk factor in and of itself would not likely lead to concerted efforts to push for widespread adoption of recommendations to have Lp(a) levels measured if a therapy for Lp(a) were not available. Indeed, proponents of evidence-based medical practice have argued against measurement of Lp(a) in the absence of effective therapy, even if one understands that this may place a given subject at higher CVD risk. Until now there has not been specific therapy and, even for the minority of patients who can tolerate relatively high doses of niacin, reductions in Lp(a) of 25–40% are usually the best that can be expected. Although newer or experimental agents such as mipomersen (22), monoclonal antibodies to PCSK9 (23), or CETP inhibitors (24) can reduce Lp(a), the extent of lowering is in general similar to or less than that achieved by niacin. However, for the first time, there appears to be a therapeutic strategy that offers the promise of both specific and effective therapy to lower plasma Lp(a). Recently, the use of antisense therapy that targets hepatic *LPA* mRNA was shown to be highly effective in reducing Lp(a) levels in humans (25). Further refinements in this methodology promise even more effective and well-tolerated therapies. Now we can begin to envision agents sufficiently specific and effective to allow clinical trials to be designed and conducted to test the efficacy of lowering Lp(a) to prevent the enhanced risk associated with CVD and CAVS.

It is against this background of exciting new data and the possible availability of specific and effective therapy that we suggest that Lp(a) is at last coming of age. In recognition of the surge in interest and new information in the Lp(a) field, we commissioned a comprehensive set of reviews of both basic and clinical aspects of Lp(a) biology to inform our readership. This issue marks the beginning of a new Thematic Series entitled "Lp(a): Coming of Age at Last". It will consist of a total of 14 articles published over the next year and authored by some of the foremost experts in Lp(a) biology. The development of efficacious therapy to lower Lp(a) levels has the potential to transform the Lp(a) field from the bench to the bedside at last. The first article in this Thematic Series, which is included in this issue, will describe the development of antisense therapy to apo(a) along with an up-to-date review of developments and expectations for this exciting therapy. This article is authored by Drs. Mark Graham, Nick Viney, Rosanne Crooke, and Sotirios (Sam) Tsimikas and is entitled "Antisense inhibition of apolipoprotein (a) to lower plasma lipoprotein (a) levels in humans." We think this pivotal development is an appropriate start to the upcoming series. The possibility to substantially and safely lower Lp(a) levels in humans suggests that at last we may be in a

position to determine the importance of Lp(a) to human biology and disease.

In the April issue, Santica Marcovina and John Albers, who have been in the forefront of efforts to develop specific and standardized measurements of Lp(a), will present a critical discussion of where we are and where we need to go to have reliable measurements of Lp(a) in their Thematic Review entitled "Lp(a) measurements for clinical application." This will be followed in May by an authoritative update on the role of Lp(a) in coagulation by Michael Boffa and Marlys Koschinsky: "Lipoprotein(a): truly a direct prothrombotic factor in cardiovascular disease?"

In June, George Thanassoulis, who led the team that made the seminal observation that Lp(a) was a genetic risk factor for CAVS, will present "Lipoprotein(a) in calcific aortic valve disease: from genomics to novel drug target for aortic stenosis." His review will bring us up to date on this fast-moving field and the implications for testing the hypothesis that lowering Lp(a) might slow the development of CAVS. In a subsequent issue, Børge Nordestgaard and colleagues, whose elegant studies in the Danish population have greatly improved our understanding of the importance of Lp(a), will review the epidemiology and genetic evidence that implicates Lp(a) in CVD and CAVS.

In coming issues, Lars Berglund and colleagues will discuss the impact of ethnicity and environmental factors that modulate Lp(a) levels. Gerd Utermann will then present a review of the interaction of genetics on the structure and function of Lp(a) and Henry Ginsberg will review what is known and what remains to be learned about Lp(a) metabolism.

Erik Stroes and colleagues will present a review on all the therapeutic options, past and present, that impact Lp(a) aside from antisense therapy, and Elisa Waldmann and Klaus Parhofer will present a detailed commentary on the role of LDL apheresis, which, for example, is now widely used in Germany to lower Lp(a) in those with existing CVD. Jemma Hopewell and Colin Baigent will review Lp(a) metabolism in renal disease and the possible role of Lp(a) in mediating the enhanced risk of CVD associated with renal failure and hemodialysis therapy. Finally, Sotirios (Sam) Tsimikas and Joseph Witztum will review what is known about the complex mechanisms by which Lp(a) promotes atherogenesis, with a particular emphasis on the emerging role of oxidized phospholipids in this pathophysiology.

Ever since the discovery of Lp(a) by Kare Berg, the Lp(a) field has moved forward as the result of contributions by many investigators, both basic and clinical. But as so often happens, there are key discoveries along the way, both those learned during the course of planned and methodical investigations, as for example, what followed the cloning of apo(a), as well as those discovered by serendipity, for example, the observation that Lp(a) was the lipoprotein carrier of OxPL, that seem to especially provide new insights and new leads for investigation. Our final contribution in the Thematic Review series will be by Gerhard Kostner, who will attempt to provide a historical overview of the Lp(a) field and to make projections into the future.

As two long-time investigators who have watched and contributed to the Lp(a) field, we are excited about the future of Lp(a) research and clinical investigation and especially about the opportunity to perhaps at last see clinical trials that will put to the test the many hypothesis on the role of Lp(a) in CVD and CAVS that have been put forth over the years. We are confident this Thematic Series will summarize the state-of-the art of the Lp(a) field and will help educate both the novice and expert who wish to gain insight into this exciting area.

REFERENCES

1. Berg, K. 1963. A new serum type system in man–the LP system. *Acta Pathol. Microbiol. Scand.* **59:** 369–382.
2. Berg, K., and J. Mohr. 1963. Genetics of the LP system. *Acta Genet. Stat. Med.* **13:** 349–360.
3. Berg, K., G. Dahlen, and M. H. Frick. 1974. Lp(a) lipoprotein and pre-beta1-lipoprotein in patients with coronary heart disease. *Clin. Genet.* **6:** 230–235.
4. Djurovic, S., and K. Berg. 1997. Epidemiology of Lp(a) lipoprotein: its role in atherosclerotic/thrombotic disease. *Clin. Genet.* **52:** 281–292.
5. Eaton, D. L., G. M. Fless, W. J. Kohr, J. W. McLean, Q. T. Xu, C. G. Miller, R. M. Lawn, and A. M. Scanu. 1987. Partial amino acid sequence of apolipoprotein(a) shows that it is homologous to plasminogen. *Proc. Natl. Acad. Sci. USA.* **84:** 3224–3228.
6. McLean, J. W., J. E. Tomlinson, W. J. Kuang, D. L. Eaton, E. Y. Chen, G. M. Fless, A. M. Scanu, and R. M. Lawn. 1987. cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. *Nature.* **330:** 132–137.
7. Koschinsky, M. L., U. Beisiegel, D. Henne-Bruns, D. L. Eaton, and R. M. Lawn. 1990. Apolipoprotein(a) size heterogeneity is related to variable number of repeat sequences in its mRNA. *Biochemistry.* **29:** 640–644.
8. Kraft, H. G., A. Lingenhel, S. Kochl, F. Hoppichler, F. Kronenberg, A. Abe, V. Muhlberger, D. Schonitzer, and G. Utermann. 1996. Apolipoprotein(a) kringle IV repeat number predicts risk for coronary heart disease. *Arterioscler. Thromb. Vasc. Biol.* **16:** 713–719.
9. Dubé, J. B., M. B. Boffa, R. A. Hegele, and M. L. Koschinsky. 2012. Lipoprotein(a): more interesting than ever after 50 years. *Curr. Opin. Lipidol.* **23:** 133–140.
10. Tsimikas, S., C. Bergmark, R. W. Beyer, R. Patel, J. Pattison, E. Miller, J. Juliano, and J. L. Witztum. 2003. Temporal increases in plasma markers of oxidized low-density lipoprotein strongly reflect the presence of acute coronary syndromes. *J. Am. Coll. Cardiol.* **41:** 360–370.
11. Bergmark, C., A. Dewan, A. Orsoni, E. Merki, E. R. Miller, M. J. Shin, C. J. Binder, S. Horkko, R. M. Krauss, M. J. Chapman, et al. 2008. A novel function of lipoprotein [a] as a preferential carrier of oxidized phospholipids in human plasma. *J. Lipid Res.* **49:** 2230–2239.
12. Erqou, S., S. Kaptoge, P. L. Perry, E. Di Angelantonio, A. Thompson, I. R. White, S. M. Marcovina, R. Collins, S. G. Thompson, and J. Danesh. 2009. Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. *JAMA.* **302:** 412–423.
13. Erqou, S., A. Thompson, E. Di Angelantonio, D. Saleheen, S. Kaptoge, S. Marcovina, and J. Danesh. 2010. Apolipoprotein(a) isoforms and the risk of vascular disease: systematic review of 40 studies involving 58,000 participants. *J. Am. Coll. Cardiol.* **55:** 2160–2167.
14. Clarke, R., J. F. Peden, J. C. Hopewell, T. Kyriakou, A. Goel, S. C. Heath, S. Parish, S. Barlera, M. G. Franzosi, S. Rust, et al. 2009. Genetic variants associated with Lp(a) lipoprotein level and coronary disease. *N. Engl. J. Med.* **361:** 2518–2528.
15. Kamstrup, P. R., A. Tybjaerg-Hansen, R. Steffensen, and B. G. Nordestgaard. 2009. Genetically elevated lipoprotein(a) and increased risk of myocardial infarction. *JAMA.* **301:** 2331–2339.
16. Kamstrup, P. R., A. Tybjaerg-Hansen, and B. G. Nordestgaard. 2012. Genetic evidence that lipoprotein(a) associates with atherosclerotic stenosis rather than venous thrombosis. *Arterioscler. Thromb. Vasc. Biol.* **32:** 1732–1741.
17. Kathiresan, S. 2009. Lp(a) lipoprotein redux–from curious molecule to causal risk factor. *N. Engl. J. Med.* **361:** 2573–2574.

18. Thanassoulis, G., C. Y. Campbell, D. S. Owens, J. G. Smith, A. V. Smith, G. M. Peloso, K. F. Kerr, S. Pechlivanis, M. J. Budoff, T. B. Harris, et al. 2013. Genetic associations with valvular calcification and aortic stenosis. *N. Engl. J. Med.* **368:** 503–512.
19. Kamstrup, P. R., A. Tybjaerg-Hansen, and B. G. Nordestgaard. 2014. Elevated lipoprotein(a) and risk of aortic valve stenosis in the general population. *J. Am. Coll. Cardiol.* **63:** 470–477.
20. Capoulade, R., K. L. Chan, C. Yeang, P. Mathieu, Y. Bosse, J. G. Dumesnil, J. W. Tam, K. K. Teo, A. Mahmut, X. Yang, et al. 2015. Oxidized Phospholipids, Lipoprotein(a), and Progression of Calcific Aortic Valve Stenosis. *J. Am. Coll. Cardiol.* **66:** 1236–1246.
21. Nordestgaard, B. G., M. J. Chapman, K. Ray, J. Boren, F. Andreotti, G. F. Watts, H. Ginsberg, P. Amarenco, A. Catapano, O. S. Descamps, et al. 2010. Lipoprotein(a) as a cardiovascular risk factor: current status. *Eur. Heart J.* **31:** 2844–2853.
22. Santos, R. D., F. J. Raal, A. L. Catapano, J. L. Witztum, E. Steinhagen-Thiessen, and S. Tsimikas. 2015. Mipomersen, an antisense oligonucleotide to apolipoprotein B-100, reduces lipoprotein(a) in various populations with hypercholesterolemia: results of 4 phase III trials. *Arterioscler. Thromb. Vasc. Biol.* **35:** 689–699.
23. Raal, F. J., R. P. Giugliano, M. S. Sabatine, M. J. Koren, G. Langslet, H. Bays, D. Blom, M. Eriksson, R. Dent, S. M. Wasserman, et al. 2014. Reduction in lipoprotein(a) with PCSK9 monoclonal antibody evolocumab (AMG 145): a pooled analysis of more than 1,300 patients in 4 phase II trials. *J. Am. Coll. Cardiol.* **63:** 1278–1288.
24. Cannon, C. P., S. Shah, H. M. Dansky, M. Davidson, E. A. Brinton, A. M. Gotto, M. Stepanavage, S. X. Liu, P. Gibbons, T. B. Ashraf, et al. 2010. Safety of anacetrapib in patients with or at high risk for coronary heart disease. *N. Engl. J. Med.* **363:** 2406–2415.
25. Tsimikas, S., N. J. Viney, S. G. Hughes, W. Singleton, M. J. Graham, B. F. Baker, J. L. Burkey, Q. Yang, S. M. Marcovina, R. S. Geary, et al. 2015. Antisense therapy targeting apolipoprotein(a): a randomised, double-blind, placebo-controlled phase 1 study. *Lancet.* **386:** 1472–1483.