Contemporary Cardiology

Series Editor: Peter P. Toth

Karam Kostner
Gerhard M. Kostner
Peter P. Toth *Editors*

Lipoprotein(a)

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Series Editor
Peter P. Toth, Ciccarone Center for the Prevention of Cardiovascular Disease
Johns Hopkins University School of Medicine
Baltimore, MD, USA

For more than a decade, cardiologists have relied on the Contemporary Cardiology series to provide them with forefront medical references on all aspects of cardiology. Each title is carefully crafted by world-renown cardiologists who comprehensively cover the most important topics in this rapidly advancing field. With more than 75 titles in print covering everything from diabetes and cardiovascular disease to the management of acute coronary syndromes, the Contemporary Cardiology series has become the leading reference source for the practice of cardiac care.

Karam Kostner • Gerhard M. Kostner
Peter P. Toth
Editors

Lipoprotein(a)

Editors
Karam Kostner
Mater Hospital
University of Queensland
Brisbane, QLD, Australia

Gerhard M. Kostner
Medical University of Graz
Graz, Austria

Peter P. Toth
Ciccarone Center for the Prevention of
Cardiovascular Disease
Johns Hopkins University School of
Medicine
Baltimore, MD, USA

ISSN 2196-8969 ISSN 2196-8977 (electronic)
Contemporary Cardiology

ISBN 978-3-031-24574-9 ISBN 978-3-031-24575-6 (eBook)
https://doi.org/10.1007/978-3-031-24575-6

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This Humana imprint is published by the registered company Springer Nature Switzerland AG
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Preface

Lipoprotein metabolism embodies great biochemical complexity and broad-spectrum functionality within serum and tissues. At first glimpse, one assumes that the role of a lipoprotein is to distribute lipids and sterols to systemic tissues and foster intermediary metabolism. Over the past five decades, we have come to learn that lipoproteins are highly active polymolecular supersystems that are extraordinarily responsive to prevailing metabolic conditions, undergo continuous modification in serum, can undergo chemical alteration when taken up into tissues, and have both beneficial and deleterious roles in health and disease. The functionality of a lipoprotein is impacted not only by its cargo of apoproteins, but also the specific constituents of its lipidome, proteome, and capacity to interact with cell surface receptors, enzymes, and intracellular signaling pathways.

Lipoprotein(a) [Lp(a)] was discovered 60 years ago and has been a biochemical and physiological enigma. It is unique among lipoproteins in that it represents a low-density lipoprotein (LDL) particle with a covalently linked apoprotein(a) moiety bound to its apoprotein B scaffold. The kringle IV repeats of the apoprotein(a) create a whole family of molecules that are genetically determined and also impact its metabolism, level in serum, and many of its molecular behaviors. A large number of clinical, epidemiological, and basic scientific investigations identify Lp(a) as highly pathogenic. Elevated levels of Lp(a) correlate with increased risk for atherosclerotic disease as well as aortic valve calcification. Like its lipoprotein cousin, LDL, it can induce endothelial cell dysfunction, potentiate adhesion molecule expression, promote the influx of inflammatory white cells into the subendothelial space of arteries, activate pro-inflammatory nuclear transcription factors, promote smooth muscle cell migration, and foam cell formation. Lp(a) activates calcium deposition proteins which can induce both aortic valve and arterial calcification. Lp(a) may also be prothrombotic. Lp(a) is an important transport vehicle of oxidized phospholipids, which can be proinflammatory, proatherogenic, and stimulate osteogenesis in various cell types.

Somewhat contrapuntal to such a diverse array of potentially injurious activity are the observations that Lp(a) participates in wound healing and angiogenesis, impacts the mortality associated with various types of cancer, participates in immunity and complement activation, is an acute phase reactant, and can modulate systemic inflammatory tone as well as risk for autoimmune disease, among other effects. Unlike other lipoproteins whose clearance from plasma is well understood, our understanding of how Lp(a) is cleared from the systemic circulation is remarkably incomplete. We do not know which receptors along the hepatocyte surface drive this process. Interestingly, although high levels of Lp(a) are predictive of heightened risk for coronary artery disease and risk of myocardial infarction, multiple longitudinal cohort studies also suggest that elevated Lp(a) levels are protective against the development of diabetes mellitus. The mechanistic basis for this finding also remains to be elucidated. Insight into the genetics of Lp(a) is progressing rapidly as is our characterization of the many Kringle IV isoforms and how their functions vary.

Lipoprotein(a) is now recognized as an important risk factor for the development of atherosclerotic disease and aortic valve stenosis. It is generally recommended that Lp(a) be measured at least once in one's lifetime for overall risk assessment. Lp(a) levels are genetically determined and, unlike the levels of other lipoproteins, generally unresponsive to lifestyle modification. Lp(a) levels are also poorly responsive to such drugs as statins, ezetimibe, fibrates, and bile acid-binding resins. Although responsive to high-dose niacin therapy, multiple trials failed to show any clinical benefit from Lp(a) reduction with this drug. Two recent trials with the use of proprotein convertase subtilisin: kexin type 9 antibodies did show that Lp(a) reduction with these molecules contributed to overall risk reduction in patients with established cardiovascular disease. The apheresis of Lp(a) also demonstrates cardiovascular benefit with reduced risk for acute coronary syndromes and death in patients with elevated Lp(a). With the dawn of ribonucleic acid therapeutics, we now have both RNA oligonucleotide and antisense technology directed against hepatic Lp(a) production. These are being tested in large prospective, randomized clinical trials to evaluate their efficacy and safety. We must also resolve how best to measure Lp(a) levels and adopt a uniform means of expressing its measured value. This is important not only for reproducible quantification, but also to make comparison between studies done in different parts of the world more feasible. Although relatively unimportant for other lipoproteins, the kidney plays a major role in Lp(a) metabolism. In the settings of chronic kidney disease and nephrotic syndrome, Lp(a) can become markedly elevated. In this volume, these issues are discussed in considerable detail.

Given all that we know and do not know about Lp(a), we thought it was time to produce a book which synthesizes what we do know about this still highly enigmatic lipoprotein, both positive and negative. We also explore unanswered

questions. While the book is highly scientific throughout, we emphasize clinical aspects whenever possible. Chapters were prepared by leading experts in the field of Lp(a) research. We anticipate that Lp(a) will emerge as a treatment target in the clinical arena and hope that this volume provides both context and knowledge that helps to ensure that clinicians will evaluate patients for Lp(a), incorporate it into cardiovascular risk stratification, and treat it as appropriate.

Brisbane, Australia Karam Kostner
Graz, Austria Gerhard M. Kostner
Baltimore, MD, USA Peter P. Toth

Contents

1 **60 Years of Lp(a) Research: From Ouchterlonys Double Diffusion to Copy Number Variation and a Significant Risk Factor for CHD** 1
Gerd Utermann

2 **Lp(a) Biochemistry, Composition, and Structure** 39
Gerhard M. Kostner

3 **Genetics of Lipoprotein(a)** 55
Gerd Utermann

4 **Lp(a) Metabolism** 75
John S. Millar and Daniel J. Rader

5 **Contemporary Aspects of Lp(a) Metabolism and Therapies Based on Tracer Kinetic Studies in Humans** 91
Dick C Chan, Jing Pang, and Gerald F Watts

6 **Role of Proprotein Convertase Subtilisin Kexin Type 9 in Lipoprotein(a) Metabolism** 113
Antonio Gallo, Kévin Chemello, Romuald Techer, Ali Jaafar, and Gilles Lambert

7 **The Role of Cell Surface Receptors in Lp(a) Catabolism** 125
Lamia Ismail, Déanna Shea, and Sally McCormick

8 **Physiological Roles and Functions of Lipoprotein(a)** 135
Zaid N. Safiullah, Thorsten Leucker, Steven R. Jones, and Peter P. Toth

9 **The Role of Lp(a) in Atherosclerosis: An Overview** 159
Anastasiya Matveyenko, Marianna Pavlyha, and Gissette Reyes-Soffer

10 Molecular Mechanisms of Lipoprotein(a) Pathogenicity: Tantalizing Clues and Unanswered Questions 173
Michael B. Boffa and Marlys L. Koschinsky

11 Thrombosis, Inflammation, and Lipoprotein(a): Clinical Implications 189
Maya S. Safarova and Patrick M. Moriarty

12 The Kidney Is the Heart of the Organs: Its Role in Lp(a) Physiology and Pathophysiology 207
Hans Dieplinger

13 Lp(a) as a Cardiovascular Risk Factor 231
Angela Pirillo and Alberico Luigi Catapano

14 Lp(a) and Aortic Valve Stenosis, Stroke, and Other Noncoronary Cardiovascular Diseases 241
Anne Langsted and Pia R. Kamstrup

15 Lipoprotein(a) in Cardiovascular Disease: Evidence from Large Epidemiological Studies 251
Peter Engel Thomas, Signe Vedel-Krogh, and Børge G. Nordestgaard

16 Lipoprotein(a) and Immunity 261
O. I. Afanasieva, T. I. Arefieva, M. V. Ezhov, and S. N. Pokrovsky

17 When Should We Measure Lipoprotein(a)? 275
Karam Kostner

18 Measurement of Lipoprotein(a) in the Clinical Laboratory 281
David Sullivan, Catherine Woolnough, Nimalie Perera, Jay Ramanathan, and Tony Badrick

19 Standardization of Analytical Methods for the Measurement of Lipoprotein(a): Bridging Past and Future Initiatives 297
Noemie Clouet-Foraison, Tomas Vaisar, and Santica M. Marcovina

20 On the Way to a Next-Generation Lp(a) Reference Measurement System Based on Quantitative Protein Mass Spectrometry and Molar Units 325
Christa Cobbaert, Liesbet Deprez, and Renee Ruhaak

21 Therapy of Elevated Lipoprotein(a) 347
S. Ibrahim and Erik S. G. Stroes

22 Antisense Oligonucleotide Therapy to Treat Elevated Lipoprotein(a) 359
Sotirios Tsimikas

23 Lipoprotein Apheresis for Reduction of Lipoprotein(a) 377
Ulrich Julius and Sergey Tselmin

24 Elevated Lp(a): Why Should I Test For It, If I Cannot Treat It? A Patient's Perspective 409
Sandra Revill Tremulis

25 Unresolved Questions 425
Gerhard M. Kostner and Karam Kostner

Correction to: The Kidney Is the Heart of the Organs: Its Role in Lp(a) Physiology and Pathophysiology C1

Index 437

Contributors

O. I. Afanasieva National Medical Research Center of Cardiology named after E. I. Chazov MOH of Russian Federation, Moscow, Russia

T. I. Arefieva National Medical Research Center of Cardiology named after E. I. Chazov MOH of Russian Federation, Moscow, Russia

Tony Badrick Royal College of Pathologists of Australasia Quality Assurance Program, St Leonards, NSW, Australia

Michael B. Boffa Robarts Research Institute, Schulich School of Medicine and Dentistry, The University of Western Ontario, London, ON, Canada

Department of Biochemistry, Schulich School of Medicine and Dentistry, The University of Western Ontario, London, ON, Canada

Alberico Luigi Catapano IRCCS MultiMedica, Milan, Italy

Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy

Dick C Chan Medical School, University of Western Australia, Perth, WA, Australia

Kévin Chemello Laboratoire Inserm UMR 1188 DéTROI, Université de La Réunion, Saint-Pierre (Ile de La Réunion), France

Noemie Clouet-Foraison Division of Metabolism, Endocrinology, and Nutrition, University of Washington, Seattle, WA, USA

Christa Cobbaert Leiden University Medical Center, Leiden, The Netherlands

Liesbet Deprez European Commission Joint Research Center (JRC), Geel, Belgium

Hans Dieplinger Department of Genetics, Institute of Genetic Epidemiology, Medical University of Innsbruck, Innsbruck, Austria

M. V. Ezhov National Medical Research Center of Cardiology named after E. I. Chazov MOH of Russian Federation, Moscow, Russia

Antonio Gallo Laboratoire Inserm UMR 1188 DéTROI, Université de La Réunion, Saint-Pierre (Ile de La Réunion), France

S. Ibrahim Department of Vascular Medicine, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

Lamia Ismail Department of Biochemistry, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand

Ali Jaafar Laboratoire Inserm UMR 1188 DéTROI, Université de La Réunion, Saint-Pierre (Ile de La Réunion), France

Steven R. Jones Ciccarone Center for the Prevention of Cardiovascular Disease, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Ulrich Julius Department of Internal Medicine III, Lipidology and Center for Extracorporeal Treatment, University Hospital Carl Gustav Carus at the Technische Universität Dresden, Dresden, Germany

Pia R. Kamstrup Department of Clinical Biochemistry, Copenhagen University Hospital-Herlev and Gentofte, Herlev, Denmark

The Copenhagen General Population Study, Copenhagen University Hospital-Herlev and Gentofte, Herlev, Denmark

Marlys L. Koschinsky Robarts Research Institute, Schulich School of Medicine and Dentistry, The University of Western Ontario, London, ON, Canada

Physiology and Pharmacology, Schulich School of Medicine and Dentistry, The University of Western Ontario, London, ON, Canada

Gerhard M. Kostner Institute of Molecular Biology and Biochemistry, Medical University of Graz, Graz, Austria

Karam Kostner Department of Cardiology, Mater Hospital and University of Queensland, Brisbane, Australia

Gilles Lambert Laboratoire Inserm UMR 1188 DéTROI, Université de La Réunion, Saint-Pierre (Ile de La Réunion), France

Anne Langsted Department of Clinical Biochemistry, Copenhagen University Hospital-Herlev and Gentofte, Herlev, Denmark

Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Thorsten Leucker Ciccarone Center for the Prevention of Cardiovascular Disease, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Santica M. Marcovina Medpace Reference Laboratories, Cincinnati, OH, USA

Anastasiya Matveyenko Division of Preventive Medicine and Nutrition, Department of Medicine, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY, USA

Sally McCormick Department of Biochemistry, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand

John S. Millar Division of Translational Medicine and Human Genetics, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Institute for Diabetes Obesity and Metabolism, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Patrick M. Moriarty Division of Endocrinology, Diabetes and Clinical Pharmacology, Department of Internal Medicine, University of Kansas Medical Center, Kansas City, KS, USA

Atherosclerosis and Lipoprotein Apheresis Center, University of Kansas Medical Center, Kansas City, KS, USA

Børge G. Nordestgaard Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Copenhagen University Hospital, University of Copenhagen, Copenhagen, Denmark

Jing Pang Medical School, University of Western Australia, Perth, WA, Australia

Marianna Pavlyha Division of Preventive Medicine and Nutrition, Department of Medicine, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY, USA

Nimalie Perera Department of Chemical Pathology, Royal Prince Alfred Hospital, Sydney Local Health District, NSW Health Pathology, University of Sydney, Sydney, NSW, Australia

Angela Pirillo Center for the Study of Atherosclerosis, E. Bassini Hospital, Milan, Italy

IRCCS MultiMedica, Milan, Italy

S. N. Pokrovsky National Medical Research Center of Cardiology named after E. I. Chazov MOH of Russian Federation, Moscow, Russia

Daniel J. Rader Division of Translational Medicine and Human Genetics, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Institute for Diabetes Obesity and Metabolism, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Institute for Translational Medicine and Therapeutics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Smilow Center for Translational Research, University of Pennsylvania, Philadelphia, PA, USA

Jay Ramanathan Department of Chemical Pathology, Royal Prince Alfred Hospital, Sydney Local Health District, NSW Health Pathology, University of Sydney, Sydney, NSW, Australia

Department of Medicine, Liverpool Hospital, Sydney South West Local Health District, Liverpool, NSW, Australia

Gissette Reyes-Soffer Division of Preventive Medicine and Nutrition, Department of Medicine, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY, USA

Renee Ruhaak Leiden University Medical Center, Leiden, The Netherlands

Maya S. Safarova Atherosclerosis and Lipid Genomics Laboratory, Mayo Clinic, Rochester, MN, USA

Department of Cardiovascular Medicine, University of Kansas Hospital and Medical Center, Kansas City, KS, USA

Zaid N. Safiullah Osler Medical House Staff, The Johns Hopkins Hospital, Baltimore, MD, USA

Déanna Shea Department of Biochemistry, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand

Erik S. G. Stroes Department of Vascular Medicine, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands

David Sullivan Department of Chemical Pathology, Royal Prince Alfred Hospital, Sydney Local Health District, NSW Health Pathology, University of Sydney, Sydney, NSW, Australia

Romuald Techer Laboratoire Inserm UMR 1188 DéTROI, Université de La Réunion, Saint-Pierre (Ile de La Réunion), France

Peter Engel Thomas Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Copenhagen University Hospital, University of Copenhagen, Copenhagen, Denmark

Peter P. Toth Ciccarone Center for the Prevention of Cardiovascular Disease, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Sandra Revill Tremulis, MBA Redwood City, CA, USA

Sergey Tselmin Department of Internal Medicine III, Lipidology and Center for Extracorporeal Treatment, University Hospital Carl Gustav Carus at the Technische Universität Dresden, Dresden, Germany

Sotirios Tsimikas Division of Cardiology, Sulpizio Cardiovascular Center, University of California San Diego, La Jolla, CA, USA

Gerd Utermann Institute for Human Genetics, Medical University of Innsbruck, Innsbruck, Austria

Institute for Genetic Epidemiology, Medical University of Innsbruck, Innsbruck, Austria

Tomas Vaisar Division of Metabolism, Endocrinology, and Nutrition, , University of Washington, Seattle, WA, USA

Signe Vedel-Krogh Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Copenhagen University Hospital, University of Copenhagen, Copenhagen, Denmark

Gerald F Watts Medical School, University of Western Australia, Perth, WA, Australia

Lipid Disorders Clinic, Department of Cardiology and Internal Medicine, Royal Perth Hospital, Perth, WA, Australia

Catherine Woolnough Department of Chemical Pathology, Royal Prince Alfred Hospital, Sydney Local Health District, NSW Health Pathology, University of Sydney, Sydney, NSW, Australia

Chapter 1
60 Years of Lp(a) Research: From Ouchterlonys Double Diffusion to Copy Number Variation and a Significant Risk Factor for CHD

Gerd Utermann

A Historical Review

At the beginning, a note of caution. A historical review by a non-historian by necessity is subjective and biased reflecting how developments in a field are perceived in retrospect by a time witness. A PubMed search for Lp(a) in March 2022 resulted in 10.330 hits. Citations therefore have to be selective. In this historical review, the author has tried to cite the first original work on a specific topic instead of a recent review but in some instance may have failed. For a comprehensive review of the Lp(a) literature until 2001, the reader is referred to Utermann (2001).

The Discovery of Lp(a)

Lipoprotein(a) was first described in 1963 by the Norwegian Physician Kåre Berg (1963) (Fig. 1.1). As frequently in science, the history of Lp(a) started with a smart idea, but ended with an unexpected result. In 1961/1962, Allison and Blumberg (Allison and Blumberg 1961; Blumberg et al. 1962) described a polymorphism of beta-lipoproteins, which they designated the Ag-system. They had observed that some sera from polytransfused patients with thalassemia contained antibodies, which distinguished between Ag-positive and Ag-negative sera from normal individuals in a test called Ouchterlonys double diffusion (Fig. 1.2). The availability of anti-Ag sera depended on luck, required testing of many patients and quality was

G. Utermann (✉)
Institute for Human Genetics, Medical University of Innsbruck, Innsbruck, Austria

Institute for Genetic Epidemiology, Medical University of Innsbruck, Innsbruck, Austria
e-mail: gerd.utermann@i-med.ac.at

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2023
K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_1

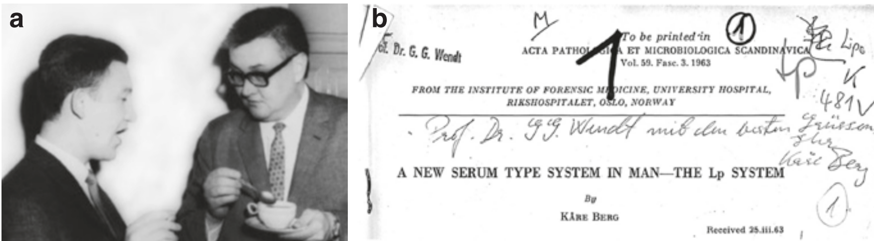


Fig. 1.1 Panel **a**: Kåre Berg and the author at the first "*International Lp workshop*" 1967 in Marburg/Lahn (Germany). Panel **b**: Title page of Kåre Berg's first publication on Lp(a) with dedication to the organizer of the workshop Gerhard G. Wendt

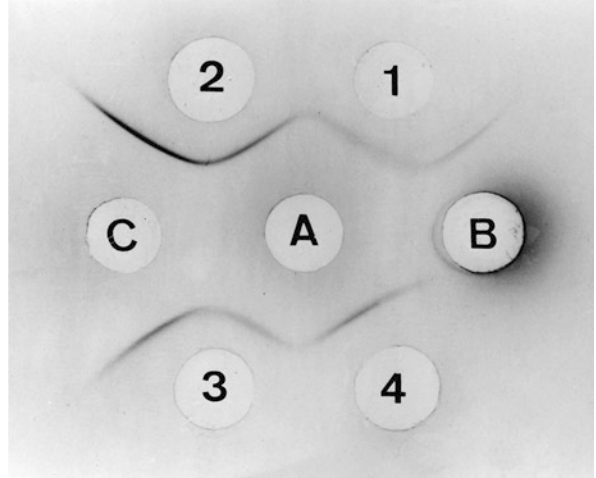


Fig. 1.2 Double diffusion in agarose gel according to Ouchterlony to test the purity of lipoprotein fractions. In wells A, B, and C, different antisera and in wells 1–4 lipoprotein fractions were applied. (A: anti-beta-lipoprotein; B: anti-lipoprotein(a); C: anti-human serum; 1: Lp(a), 2: beta-lipoprotein; 3: Lp(a); 4: beta-lipoprotein)

difficult to control. Because antisera from polytransfused patients were not readily available, limited in quantity, and could not be reproduced in the laboratory Kåre Berg, at the time at the Institute of Forensic Medicine, Rikshospitalet, University of Oslo/Norway had an idea to overcome these limitations. If an antigen elicited an immune response in humans, it should do so also in rabbits. He started a series of experiments, in which he immunized rabbits with individual human sera or beta-lipoproteins. The rabbit sera were then "absorbed" with different individual human sera to remove antibodies against foreign antigens present in all human sera. Subsequently, the absorbed rabbit sera were tested against a panel of human sera for antibodies recognizing individual human sera. Indeed, the plan worked. Some rabbit antisera reacted positive with some human sera and negative with others in Ouchterlonys double diffusion (Fig. 1.2). Moreover, Berg could show that the

antigen in fact was a beta-lipoprotein. However, it was not identical with the Ag-antigen (Berg 1964). Therefore, Berg introduced the name "Lp-System," which was later changed in Lp(a) to distinguish Lp(a) from other "Lp" Antigens. One was "Lp(x)" which was detected by antisera produced in horse but turned out to be an artifact. Berg distinguished between Lp(a+) and Lp(a−) individuals and showed by family studies that the Lp(a) trait seemed to follow an autosomal dominant mode of inheritance (Berg and Mohr 1963). Soon following the breakthrough discovery of Berg, several laboratories tried to reproduce his finding, but with mixed results. In principle, all confirmed Berg's findings, but several researchers noticed that with their antibodies the immune reaction was not an all or none. Instead, they observed strong reactions (Bergs positives), no reactions (Bergs negatives), but also weak and very weak reactions. It followed a discussion on whether the weak reactions were true Lp(a) reactions or whether the antisera which recognized weak reactions were unspecific containing antibodies to other components.

To clarify the situation and exchange latest research results, the human geneticist Gerhard G. Wendt initiated the first "International Lp workshop" in Marburg/Lahn, Germany (Wendt 1967). In preparation of the conference researchers from six different laboratories, including Kåre Berg's sent in 17 antisera, which were tested against a standard panel of 71 individual sera and analyzed for identity. The result was that all antisera recognized the same antigen Lp(a), but confirmed the existence of weak and very weak reactions which occurred to different degrees depending on the antiserum. The issue was only resolved when researchers developed methods to semi-quantify and finally quantify Lp(a), which demonstrated that Lp(a) in fact is a quantitative trait (Harvie and Schultz 1970; Ehnholm et al. 1971). Methods to quantify Lp(a) demonstrated large differences in median Lp(a) levels between and within major human ethnic groups. The distributions of Lp(a) levels were highly skewed in European and East-Asian populations but less so in sub-Saharan Africans (Fig. 1.3). Mean and median Lp(a) levels were two to fourfold higher in Africans than Europeans. The distributions in Asian populations were heterogenous with higher Lp(a) levels in South-East Asia (Sandholzer et al. 1991; Parra et al. 1987a; Helmhold et al. 1991).

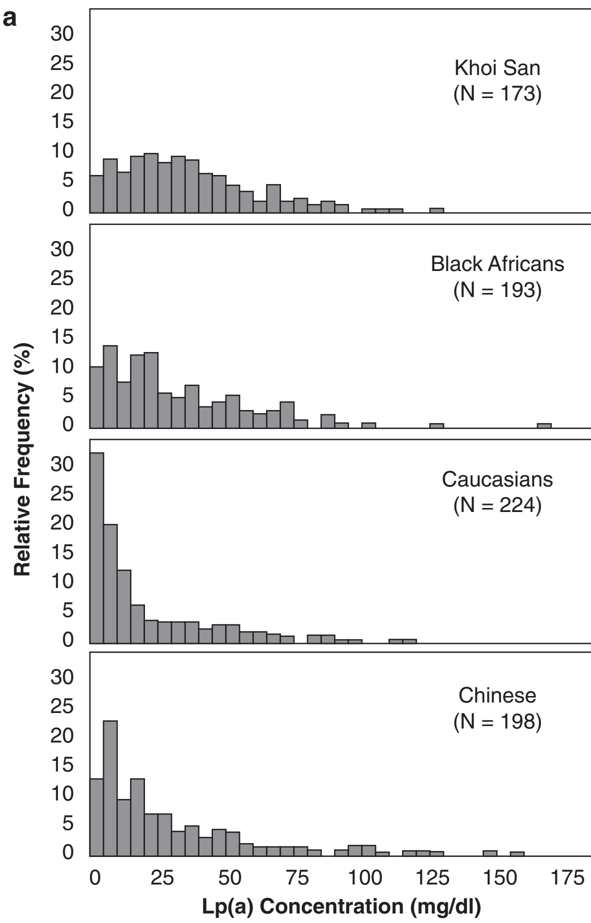
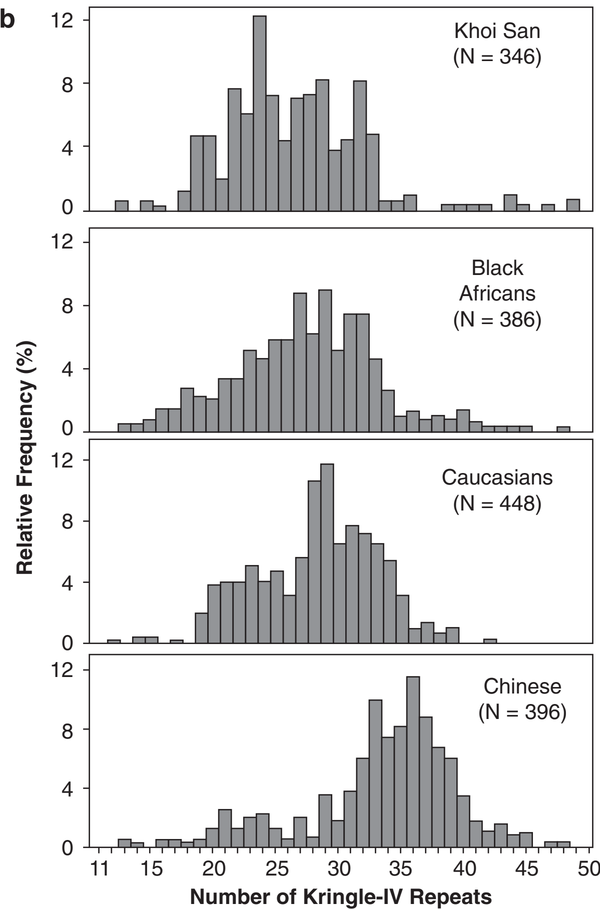


Fig. 1.3 Histograms showing: Panel (**a**) the distribution of plasma Lp(a) levels in four populations. Panel (**b**) the frequency distribution of the KIV-2 VNTR alleles in the same populations. The total number of KIV repeats including the "unique" kringles is given. Panel (**c**) the inverse correlation of KIV-2 repeats with Lp(a) concentration in the four populations. The Black Africans in this study were from South Africa and represent different ethnicities. The Chinese samples were from Hongkong and the "Caucasians" from Austria. (Figure reproduced from Kraft et al. 1996b with permission)



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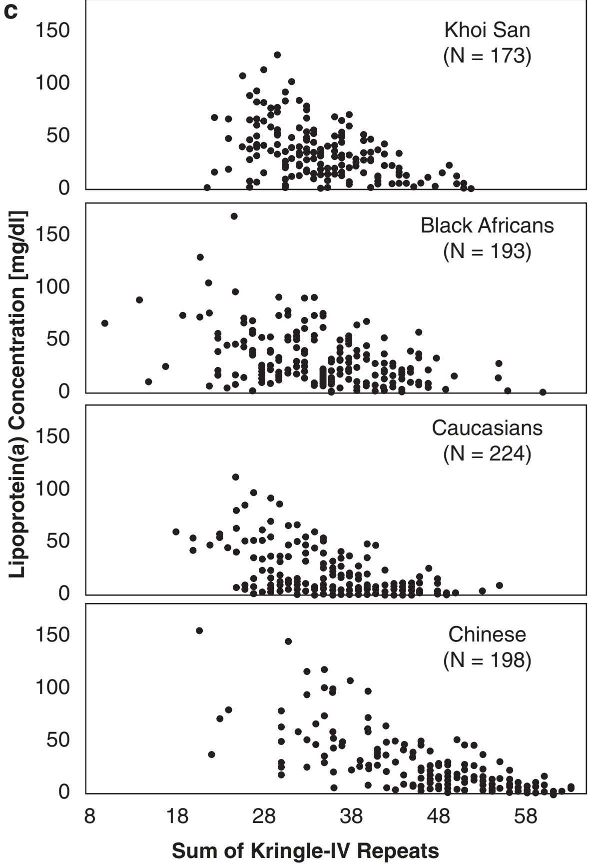

Fig. 1.3 (continued)

Isolation and Characterization of Lp(a)

Beginning in 1968, first attempts were made to isolate Lp(a) from plasma and it was shown that the antigenic property of Lp(a) is associated with a lipoprotein distinct from LDL (Wiegandt et al. 1968; Utermann and Wiegandt 1969; Schultz et al. 1968).

A major breakthrough in Lp(a) research was the purification and characterization of Lp(a) in 1970 by Christian Ehnholm (Fig. 1.4) in Kai Simons group in Helsinki, Finland. They purified Lp(a) by a combination of preparative ultracentrifugation and gel filtration on Sepharose 2B/4B columns and determined the physicochemical properties of the particle (Ehnholm et al. 1971; Simons et al. 1970). Characteristics of Lp(a) were a hydrated density of 1.09 g/mL, a molecular weight estimated by gel filtration of 4.8 MDa and by electron microscopy of 5.6 MDa. Lp(a) had pre-beta mobility in agarose gel electrophoresis and appeared as a spherical particle upon electron microscopy. Notably it differed from LDL in amino acid composition and

Fig. 1.4 Panel (**a**) Christian Ehnholm during a visit in Marburg/Lahn 1971 with the author. Panel (**b**) laboratory equipment with Sepharose 4B column (red arrows) for final purification of Lp(a) according to Ehnholm et al. (Ehnholm et al. 1971; Simons et al. 1970)

contained a very high amount of protein-bound carbohydrate. In further work, they characterized the carbohydrates in more detail and found that Lp(a) contains an about six times higher amount of sialic acid, a three times higher content of hexosamines and twice as much hexoses than LDL (Ehnholm et al. 1972). The antigenic property of Lp(a) and the high carbohydrate content were associated with a protein which occurred as a separate band in polyacrylamide gel electrophoresis, when Lp(a) disaggregated spontaneously upon storage at 0 °C (Ehnholm et al. 1972; Utermann et al. 1972). The final purification of Lp(a) by Sepharose 4B column chromatography required near-acrobatic skills. Christian Ehnholm had introduced us into the handling of the column when he visited our lab in Marburg in 1971 (Fig. 1.4). The dimension of the glass column which had to be filled with the Sephadex slurry by hand was 2.5 cm (diameter) × >160 cm (height). We used a ladder to fill the column and apply the lipoprotein sample.

The availability of a standardized reproducible method to purify Lp(a) allowed its further characterization. By SDS-polyacrylamide gel electrophoresis under reducing and non-reducing conditions and immunochemical methods, it was shown that Lp(a) contains two high molecular weight proteins, apolipoprotein B [apoB] identical with apoB in LDL (MW about 500 kDa) and a glycoprotein with a MW (about 600 kDa) which appeared to be even larger than apoB. Both proteins were hold together by one or more disulfide bonds (Utermann and Weber 1983; Gaubatz et al. 1983).

Lp(a) is usually depicted in cartoons as a global particle with an LDL in its center and apo(a) wrapped around. Studies by electron microscopy of negatively stained Lp(a) (Sines et al. 1994) and small-angel X-ray scattering (Prassl et al. 1995) supported this model but other studies suggested that apo(a) may protrude as a "tail" from the particle depending on the environment (Weisel et al. 2001) and that Lp(a) can switch between globular and "flexible tail" structures (Becker et al. 2004).

Metabolism

Studies of the metabolism of Lp(a) started in 1979 when Gerhard Kostner's group in Graz/Austria initiated a series of in vivo turnover studies in humans (Krempler et al. 1979, 1980, 1983). Healthy individuals with different concentrations of Lp(a) were injected with radioiodinated Lp(a) and in subsequent experiments with radioiodinated LDL. They demonstrated that (1) Lp(a) is not a metabolic product of other apoB-containing lipoproteins, (2) that Lp(a) concentrations in plasma are determined by the rate of synthesis rather than by its catabolism, and (3) that Lp(a) is catabolized at a slower rate than LDL.

Binding studies of radioactively labeled Lp(a) to human fibroblasts in comparison with LDL confirmed results of Havekes et al. (1981) and demonstrated that Lp(a) binds with high affinity to the same cell surface receptor as LDL. However, binding capacity for Lp(a) was lower than for LDL (Krempler et al. 1983). Lp(a) did not bind to fibroblasts from patients with homozygous FH. These findings are in line with later binding studies in fibroblasts and experiments in transgenic mice by Goldstein and Brown (Hofmann et al. 1990) which indicated that Lp(a) is removed from plasma by the LDL receptor pathway and with the observation that patients with FH due to LDL receptor mutations or apoB100 mutations have elevated Lp(a) in plasma (Seed et al. 1990; Utermann et al. 1989; Lingenhel et al. 1998; Van der Hoek et al. 1997; Kraft et al. 2000). Together, these findings give a consistent picture. However, later studies on these topics were highly controversial and neither confirmed the binding of Lp(a) to fibroblasts, nor the in vivo turnover studies in humans and transgenic mice or the family studies (Soutar et al. 1991; Knight et al. 1991; Cain et al. 2005; Rader et al. 1995). Other receptors and pathways were implicated to be involved in the removal of Lp(a) from plasma (reviewed in McCormick and Schneider 2019). The liver (Cain et al. 2005) and the kidney (Kronenberg et al. 1997) both have been suggested as major sites of Lp(a) clearance from plasma. A role for the kidney in Lp(a) clearance from the circulation was championed by the group of Florian Kronenberg and Hans Dieplinger in Innsbruck and is supported by several lines of evidence. Turnover studies demonstrated a reduced clearance of Lp(a) in patients with kidney disease (Frischmann et al. 2007). Large arteriovenous differences between Lp(a) concentrations were observed in the renovascular system (Kronenberg et al. 1997). A problem with this study is that it requires the assumption of unreasonably high synthesis rates of Lp(a) to compensate for the loss in the kidneys. Further, Lp(a) binds with high affinity to megalin/gp330, a member of the

LDLR family expressed preferentially in kidneys (Niemeier et al. 1999) and fragments of apo(a) were found in human urine (Oida et al. 1992; Mooser et al. 1996; Kostner et al. 1996). None of this is direct evidence and at present the tissue(s) and pathways of Lp(a) removal from the circulation remain unresolved. In contrast, the liver as the site of synthesis and secretion of Lp(a) is undisputed. Hans-Georg Kraft and colleagues from Innsbruck determined apo(a) isoform phenotypes (see below) in plasma from patients undergoing liver transplantation and their organ donors. They observed that genetic isoform phenotypes changed completely from recipients to the donors phenotype following transplantation (Kraft et al. 1989). Apo(a) mRNA was also most abundant in the liver from rhesus baboons and cynomolgus monkeys (Tomlinson et al. 1989; Hixson et al. 1989; Azrolan et al. 1991).

The LPA Gene and Apolipoprotein(a)

Two important discoveries were made in 1987, the unique structure of apo(a) (McLean et al. 1987) and the isoform polymorphism of apo(a) (Utermann et al. 1987).

The sequence of apo(a) had remained elusive for a long time and the reason became clear when the sequence was finally resolved. Attempts to determine the amino acids sequence of apo(a) by protein sequencing resulted in partial amino acids sequences which demonstrated a high homology to plasminogen (Eaton et al. 1987; Kratzin et al. 1987). Only by the breakthrough work of Richard Lawn and colleagues, at that time working at Genentech, the full sequence was elucidated. As a pioneer in cloning technologies and DNA sequencing, Richard Lawn (Fig. 1.5) who had previously sequenced hemoglobin loci from thalassemia patients started cloning and cDNA sequencing of the *LPA* gene. This turned out to be much more complicated than previous work. The result was unanticipated and astonishing. The deduced amino acid sequence of apo(a) consisted of an array of so-called kringle domains with a high internal homology and homology to kringle 4 from plasminogen. Ten

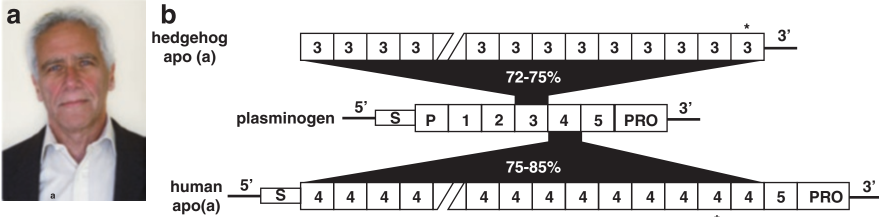

Fig. 1.5 Panel (**a**) Richard M. Lawn who published the first cDNA sequence of apo(a) (McLean et al. 1987). Panel (**b**) Illustration of the convergent evolution of primate and insectivore apo(a). The cDNA structures of plasminogen and human and hedgehog apo(a) are shown. Kringle types are denoted by numbers and the protease domain by PRO. The stars indicate the sites of the unpaired Cys residues which form the disulfide bridge with apoB in LDL. The percentages give the degree of homology between plasminogen and apo(a). (Reproduced from Lawn et al. 1995b with permission)

kringle type IV different in sequence was identified, nine of them in single copy (KIV-1, KIV-3 to KIV-10) whereas one (KIV-2) occurred in six identical copies in the sequenced DNA. In addition, the protein contained one kringle with homology to KV from *PLG*, a signal sequence and a plasminogen-like protease domain. The latter was predicted to be inactive toward plasmin substrates due to mutation in the catalytic triad. The findings showed that the *LPA* gene had been derived from the *PLG* gene during evolution by a number of changes including duplication of *PLG*, deletions and expansions of domains and mutations. The structure of all kringles is stabilized by three internal disulfide bridges, which results in the typical appearance of a Danish bretzel called "kringle." One kringle (KIV-9) in addition contains one unpaired cys residue, which turned out to be responsible for the covalent binding to apoB of the LDL particle (Koschinsky et al. 1993; Brunner et al. 1993).

The sequence was so unusual that in an accompanying "News and Views" article in Nature Joseph Goldstein and Michael Brown wrote that "*the…. finding challenges the notion that evolution makes sense*" (Brown and Goldstein 1987). The enormous challenge which sequencing of *LPA* posed at the time becomes evident when one considers that a successful search for mutations in the KIV-2 repeats of *LPA* became possible only very recently (Coassin et al. 2019). It was certainly the most heroic undertaking in Lp(a) research and opened new unexpected avenues.

Sequence analysis of *PLG* and human and rhesus *LPA* (Tomlinson et al. 1989) had shown that *LPA* had evolved from *PLG* after the split of Old-world from New-world monkeys some 40 million years ago and that Lp(a) existed only in Old-world monkeys. Therefore, it was a surprise when Laplaud et al. in France reported presence of Lp(a) in the plasma of a hibernator, the hedgehog (Laplaud et al. 1988). The surprise became even bigger when Lawns group sequenced the *LPA* of hedgehog. Instead of KIV repeats, it contained multiple copies of KIII as the sole kringle type and lacked the protease domain (Lawn et al. 1995a, 1997), but like the primate counterpart hedgehog apo(a) formed a Lp(a) particle with LDL. Reports on the presence of Lp(a) in guinea pigs (Rath and Pauling 1990) and the marmoset (a New-world monkey) (Guo et al. 1991) were not confirmed. Hence, the occurrence of a Lp(a)-like particle in the hedgehog by convergent evolution apparently remained a solitary act (Lawn et al. 1995a, 1997).

Functional Studies

As shown by the work of Lawn and colleagues, the *LPA* gene had evolved from *PLG* during primate evolution suggesting that the function of Lp(a) might be related to the function of plasminogen and blood clotting. Already in the "News and Views" article mentioned above, Goldstein and Brown had put forward the hypothesis that Lp(a) through binding to fibrinogen might be involved in wound healing (Brown and Goldstein 1987). The hypothesis—which still appears attractive, but was never rigorously tested though—considers that Lp(a) is a macromolecular complex containing two very different components, apo(a) and LDL. For most functions assigned

to Lp(a), the apo(a) alone is sufficient which leaves open the question, why the particle exists. Beginning with the work of Harpel et al. (1989, 1995), numerous studies demonstrated effects of Lp(a) on the blood clotting cascade and connected thrombosis to atherosclerosis (Nachman 1992). Lp(a) was described as "an interloper into the fibrinolytic system" (Miles and Plow 1990). Several interactions of apo(a) with diverse ligands have been reported (Fig. 1.6) but whether any is of physiological or pathophysiological relevance in humans remains unclear. A prominent hypothesis explaining the atherogenic potential of Lp(a) was derived from the finding that it is a "sink" for oxidized phospholipids (Tsimikas et al. 2005; Kiechl et al. 2007; Bergmark et al. 2008). Lack in understanding of the function and pathophysiological properties have recently been reviewed by an NHLBI working group (Tsimikas et al. 2018). Existence of Lp(a) and the apo(a) size polymorphism in Old-world monkeys implies that Lp(a) may have, or had, a function beyond one species. The detection of many null mutations in the *LPA* gene (see below) may, however, indicate that this function has been lost in modern humans with the possible exception of Africans.

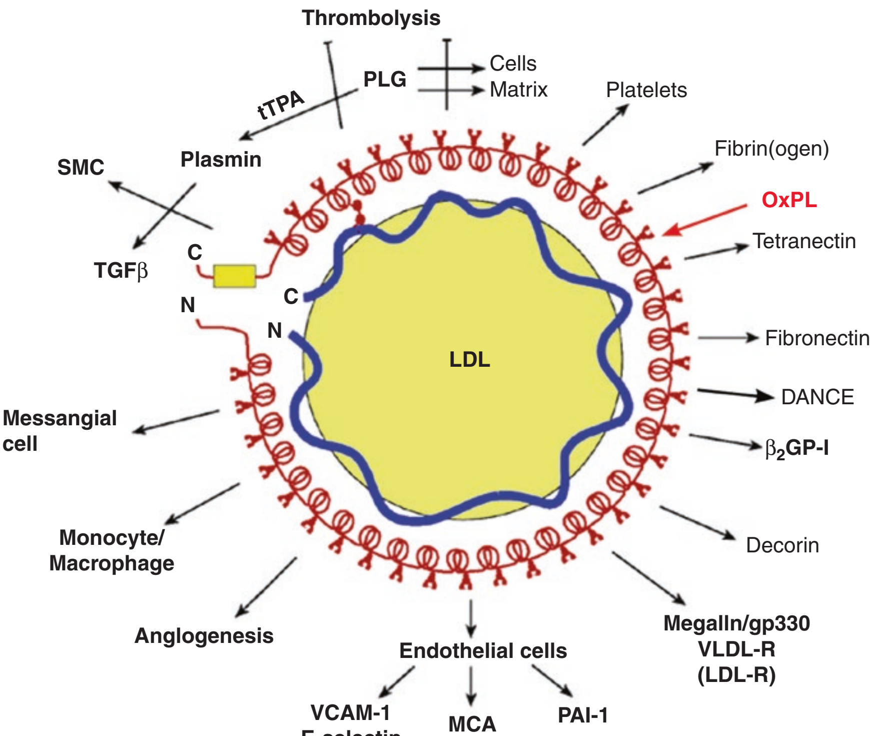

Fig. 1.6 Model of Lp(a) and reported interactions of Lp(a)/apo(a) with components of the blood clotting system, cell receptors, and other binding proteins. The binding of oxidized phospholipids (OxPL in red) is considered as crucial for the pathogenicity of Lp(a). For explanation, see text and Schmidt et al. (2016). (Modified from Utermann 1989, 2001 with permission)

Animal Studies and the Era of Transgenics

With the exception of the hedgehog, Lp(a) exists only in Old-world monkeys and humans (Makino et al. 1989). The availability of natural animal models to study the metabolism and pathophysiology of Lp(a) is limited. Rainwater and colleagues analyzed the genetics of Lp(a) extensively in baboons (Rainwater et al. 1986; White et al. 1994a) and few studies were performed in rhesus monkeys (Rudel et al. 1977; Williams-Blangero and Rainwater 1991; Enkhmaa et al. 2015) and more recently in chimpanzees (Noureen et al. 2017). The isoform polymorphism and the inverse correlation between isoform size and Lp(a) levels in plasma existed in all these primates. Chimpanzees from different West-African and Central-African habitats had significantly different Lp(a) levels and isoform distributions in plasma (Noureen et al. 2017). Experimental studies with these species are not allowed and unethical.

Therefore researchers started to generate mice transgenic for apo(a) immediately following the cloning of apo(a) cDNA (Chiesa et al. 1992). The first animals generated had apo(a) free in plasma because mouse LDL apparently lacked the structural requirement for binding apo(a) and forming the Lp(a) complex. Different approaches were used to overcome this. Infusion of human LDL into apo(a) transgenic mice resulted in the association of secreted apo(a) with circulating LDL and formation of Lp(a) which could only be resolved by disulfide reduction (Chiesa et al. 1992). In another study, human Lp(a) was infused into mice transgenic for the human LDL receptor which confirmed cell culture studies which had shown high-affinity binding of Lp(a) to the receptor (Hofmann et al. 1990). With such short-term experiments, it was not possible to investigate the pathophysiology and in particular the atherogenic potential of Lp(a). This became possible when apo(a) transgenic mice were crossed with mice strains transgenic for human apoB (Linton et al. 1993; Callow et al. 1994). These mice strains were used to study the assembly (Callow et al. 1994; Callow and Rubin 1995) and the atherogenic potential of Lp(a) (Callow et al. 1995; Mancini et al. 1995a). To identify sequence elements that regulate liver-specific tissue expression, sex hormone and diet response mice transgenic for yeast artificial chromosomes (YACs) containing entire human apo(a) alleles were produced (Frazer et al. 1995; Acquati et al. 1999).

Unraveling the Genetics of Lp(a)

The second important finding in 1987 was the discovery of the size polymorphism of apo(a). Beginning with its detection, it was clear that Lp(a) was a genetic trait. Family and twin studies had shown that heritability of the trait is high. Morton et al. (1985) concluded from a large family study that Lp(a) levels are controlled by one major dominant gene and a residual heritable component. The gene(s) controlling Lp(a) levels were unknown. This started to change when a group in Innsbruck/Austria demonstrated that several genetic isoforms of apo(a), which differ in size,

occur in the population and that the size of isoforms correlated inversely with plasma Lp(a) concentrations (Utermann et al. 1987; Utermann 1989), suggesting that Lp(a) concentrations might be controlled by the *LPA* locus, which was confirmed by subsequent sib–pair linkage studies in European and North-American White families (Boerwinkle et al. 1992; Kraft et al. 1992; Demeester et al. 1995; Scholz et al. 1999) and in African Americans (Mooser et al. 1997). Sib–pair linkage studies in families from South Africa and from Gabon demonstrated that the *LPA* locus is the major locus determining Lp(a) levels also in autochthonous populations from sub-Saharan Africa (Scholz et al. 1999; Schmidt et al. 2006). Compared to populations of European descent, the KIV-2 VNTR explained less of the variation in Lp(a) levels in Africans.

The size polymorphism was detected with the at that time new technique of Western blotting. This method had, however, a drawback. The intensity of isoforms varied widely depending on the associated Lp(a) concentrations. Many individuals exhibited only one isoform upon Western blotting. For those individuals, it was unclear whether they were homozygotes, i.e., expressed two isoforms of identical size or whether one isoform was below detection limit or due to a none-expressed allele (so-called null alleles). DNA technology was the way to overcome the problem. Already, the DNA sequence data demonstrating multiple identical copies of kringle IV-2 had Lawn and colleagues led to speculate that differences in repeat number might underlay the size polymorphism of apo(a). Semiquantitative data from Southern blotting using a KIV-2-specific sequence as probe (Utermann 1989; Lindahl et al. 1990) and differences in length of apo(a) mRNA from liver (Koschinsky et al. 1990) supported this. The application of pulse-field gel electrophoresis/ Southern blotting, which had started as a collaboration and ended in a race, finally allowed the group of Helen Hobbs in Dallas (Boerwinkle et al. 1992; Lackner et al. 1991) and Hans-Georg Kraft and colleagues in Innsbruck (Kraft et al. 1992) to demonstrate the size polymorphism at the DNA level. By using appropriate nucleases (e.g., KpnI), which cut the DNA only outside the KIV-2 sequence, allowed to retain the entire repeat block in large DNA fragments of 20 to >200 kb. Its size could be finally determined by PFGE/Southern blotting. It turned out that the protein size polymorphism resulted from a transcribed and translated copy number variation. The genomic size of one KIV-2 copy was 5.6 kb. Today only a few protein coding VNTRs have been characterized including in the *PMU* genes (Swallow et al. 1987), human proline-rich protein (Lyons et al. 1988), and the gene coding for length variation in the keratin 10 chain (Korge et al. 1992). Very recently, these transcribed and translated genes including *LPA* were identified in a genome- and exomewide search (Mukamel et al. 2021). *LPA* is the most extensively studied with a large impact on human health (Schmidt et al. 2016; Kronenberg 2016). In particular, Helen Hobbs and colleagues in Dallas characterized the *LPA* locus in detail at the molecular level (Lackner et al. 1991, 1993).

The analysis by PFGE/Southern blotting alone, however, also resulted in an incomplete picture. Whether and to which extend an allele was transcribed and translated into protein could not be seen. Only the simultaneous application of PFGE/Southern blotting of DNA and Western blotting of plasma allowed a

comprehensive characterization of *LPA* alleles by KIV-2 copy number, isoform size, and associated Lp(a) concentration (allele-associated Lp(a) concentration.

It remained however unclear whether the association of the KIV-2 VNTR with Lp(a) concentrations reflected a causal relationship and—if so—what the mechanism might be. Studies in transfected liver cell cultures from humans and in primary liver cells from baboons demonstrated that post-translational processing of apo(a) is the major determinant of Lp(a) concentrations (White et al. 1994a, b; Brunner et al. 1996; Lobentanz et al. 1998). No apo(a)-apoB complexes were detected inside the cells, but only in the cell culture media indicating that Lp(a) assembly takes place outside cells at the plasma membrane, in the space of Disse or in plasma following the separate secretion of apo(a) and LDL. This view is supported by transfusion of LDL into the plasma of apo(a) transgenic mice (Chiesa et al. 1992) and studies by Marlys Koschinsky's group who demonstrated extracellular formation of the disulfide bond between cys4326 of apoB in LDL (Callow and Rubin 1995) and the free cys residue in KIV-9 of apo(a) (Koschinsky et al. 1993; Becker et al. 2006).

By sib-pair analysis using genotypes defined by PFGE/Southern blotting, it was demonstrated that 70–95% of the variability in Lp(a) concentrations in the population is determined by the *LPA* locus. Together with earlier observations that isoforms of the same size are associated with a wide range of Lp(a) concentrations, this implied that sequence variation in *LPA* in addition to the CNV determines Lp(a) concentration. Such variation was identified in the form of restriction site polymorphisms in the KIV-2 repeat (Mancini et al. 1995b) a pentanucleotide polymorphism (PNRP) (Mooser et al. 1995; Trommsdorff et al. 1995) and a +93 C/T polymorphism (Zysow et al. 1995; Kraft et al. 1998), which explained some of the variation in Lp(a) concentrations independent from the KIV-2 VNTR. Hence, at the end of the 1990th the genetics of the Lp(a) trait was in principle clarified. The *LPA* locus was identified as the major locus for Lp(a). The two alleles at the locus defined by KIV-2 copy number and sequence variation determine Lp(a) levels in an individual and the frequency distribution of alleles determine the distribution of Lp(a) concentrations in a population (Utermann 1999). However, the details of the genetic architecture of the Lp(a) trait remained to be solved, i.e., the types of sequence variation, frequencies of SNPs, LDs with copy numbers, effect size on Lp(a) levels, etc., had to be determined. Sequence variation described to this point with one possible exception (Zysow et al. 1995; Kraft et al. 1998) had no proven direct causal effect on Lp(a). In a next step, an attempt was made to find likely causal variation in *LPA* by improved mutation screening and sequencing techniques. Some variants were detected most of which were silent and only one, a Thr>Pro substitution in position 12 of KIV-8 (identical with KIV-8 T23>P in Ogorelkova et al. 2001) was associated with Lp(a) levels but functional studies supporting causality were lacking (Prins et al. 1997, 1999). Ogorelkova and colleagues in Innsbruck analyzed the "unique" kringles 6–10 in *LPA* in different ethnic groups by the mutation screening technology denaturing gradient gel electrophoresis (DGGE) and subsequent Sanger sequencing of aberrant fragments. They were the first to identify several single nucleotide polymorphisms (SNPs) in the unique kringles of *LPA*, which resulted in amino acid substitutions and splice site variation which were strongly associated

with Lp(a) levels (Ogorelkova et al. 1999, 2001). With one exception, the SNPs were not shared between populations (Fig. 1.7). The splice site SNP (Ogorelkova et al. 1999) was shown by expression experiments in cell culture to result in a truncated apo(a) protein unable to form the Lp(a) complex. This null allele had a frequency of 0.053 in Tyrolians from Austria and 0.0635 in the Finnish population. It was rediscovered in a large population genetic study in Finns (Lim et al. 2014) without reference to the previous work. A high number of homozygotes was identified which had no associated clinical symptoms which led the authors to conclude that Lp(a) has no essential function in vivo which is amazing for results from a study analyzing an isolated population. Parson et al. (2004) identified a mutation in the KIV-2 region of *LPA* which resulted in a stop codon (R21X) and extremely low allele-associated Lp(a) levels. Later, large-scale studies showed that this variant has a carrier frequency between 1.6% and 2.1% in European populations; 1000 Genome data found that the R21X variant mostly occurs in Europeans and South Asians, is absent in Africans, and shows varying frequencies in South American populations (Di Maio et al. 2020).

A resequencing study of *LPA* was performed by the group of Crawford in individuals of non-Hispanic black and white ancestry from North America (Crawford et al. 2008). Nineteen of the identified SNPs were then analyzed for an association with plasma Lp(a) levels in >7000 participants of a population-based survey which

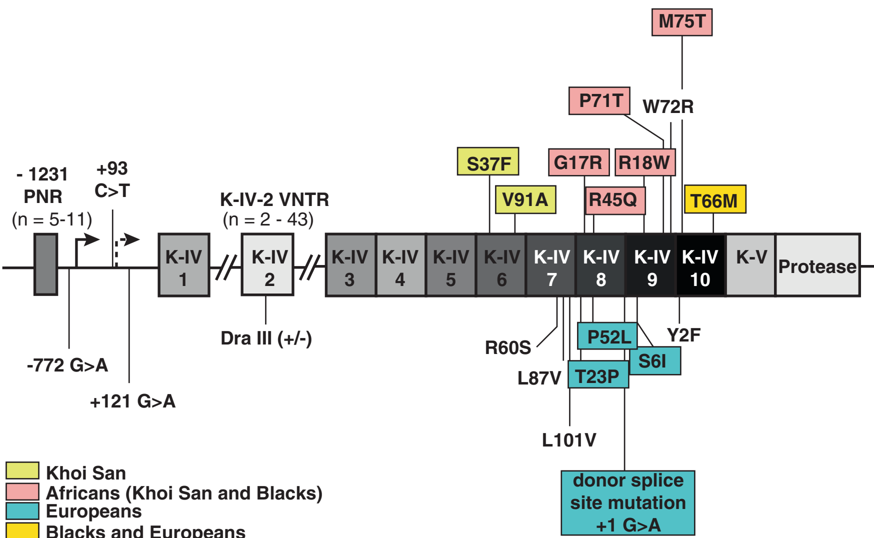

Fig. 1.7 Figures illustrating differences in the genetic architecture of the Lp(a) trait between human ethnic groups. The structure of the *LPA* gene with variants known until 2001 is shown. Data compiled from Ogorelkova et al. (2001), Prins et al. (1999), Scanu et al. (1994). The color code is only used for variants detected in the study including all four populations (Ogorelkova et al. 2001) and illustrates that many variants in *LPA* occur only in one or few populations which is in agreement with later larger studies (Mukamel et al. 2021; Dumitrescu et al. 2011). (Modified from Utermann 1999 with permission)

included three ethnic groups: Mexican Americans and non-Hispanic blacks and whites (Dumitrescu et al. 2011). They found 15 SNPs which were associated with Lp(a) levels in at least one ethnic group but none in all groups. They were not in strong LD with the KIV-2 VNTR and explained from 7% to 11% of the variance of Lp(a) levels in the respective ethnic group. Four of the variants were predicted by PolyPhen to be possibly or probably damaging, but no functional studies were performed. One variant KIV-8 Thr>Pro had also been described by Ogorelkova et al. (2001). Hence, this large study extended and confirmed previous work. Very recently, Sally McCormick's group in New Zeeland performed a detailed functional analysis of two non-synonymous variants R990Q and R1771C, which had been detected in GWAS. They showed that both are causative for null Lp(a) phenotypes and occur in positions homologous to positions in *PLG* which when mutated result in *PLG* deficiency (Morgan et al. 2020). These are the first functionally characterized non-synonymous null mutations in the *LPA* gene.

With the exception of the one identified deleterious SNP R21X (Parson et al. 2004), the KIV-2 VNTR remained a black box for mutation detection until very recently, when Asma Noureen and colleagues specifically amplified the KIV-2 target region by PCR from 90 PFGE-separated alleles from Asian, European, and four different African populations and identified several SNPs in populations of African, Asian, and European ancestry by Sanger sequencing (Noureen et al. 2015). As reported for many other genes and from genome sequencing, they observed a higher frequency of variable sites in Africans. Two previously unreported splice site variants were detected. One was a true null allele with no detectable Lp(a) associated and the other had a high frequency (10–40%) in Africans. Their approach had the advantage that SNPs could be assigned to KIV-2 copy number, but the disadvantage that sensitivity was low and mutation detection was limited and depended on copy number and on the number of KIV-2 repeats carrying the variant (intra-allelic frequency). These problems were overcome when Stefan Coassin and colleagues in Florian Kronenberg's group in Innsbruck developed deep sequencing protocols, which allowed systematic high-throughput mutation analysis of the KIV-2 VNTR (Coassin et al. 2019). They identified a variety of new variants in *LPA* and analyzed the effects on Lp(a) and associations with CHD of previously known (Di Maio et al. 2020) and newly identified variants (Coassin et al. 2017, 2020; Schachtl-Riess et al. 2021) in great detail. Their work and very recent genomic analysis (Mukamel et al. 2021) are presently at the cutting edge of Lp(a) genetics research. In particular by the genomic analysis of Mukamel et al. (2021), many gaps in our knowledge of the genetic architecture of the Lp(a) trait have been filled. They estimated KIV-2 VNTR length from whole-genome sequencing data and defined VNTR alleles by imputation of SNP data which allowed to estimate frequencies and effects of VNTR haplotypes on Lp(a) levels in populations of African, Asian, and European ancestry. A total of 17 protein-altering variants each of which reduced Lp(a) levels significantly as well as variants in the 5′ UTR which increased Lp(a) levels were observed. Previously, the variants responsible for inter-population differences were largely unknown. SNPs which had been claimed to explain level differences between ethnic groups (Deo et al. 2011; Chretien et al. 2006) do this in a statistical sense only with

a few exceptions (Coassin et al. 2017; Schachtl-Riess et al. 2021). Mukamel et al. (2021) now reported highly significant differences in the frequencies of variants with causal effects between major human ethnic groups. This explains much of the inter-ethnic differences in the genetic architecture of the Lp(a) trait between these groups.

In addition to the major *LPA* locus, other genes have been identified which make minor contributions to the variability of Lp(a) level variation including *APOE* (De Knijff et al. 1991; Klausen et al. 1996) and *APOH*/ß2GPI (Hoekstra et al. 2021). Genetic variation, which is restricted to an ethnic group, may also contribute. An example is PCSK9. Loss-of-function mutations in this gene lower Lp(a) levels in American blacks (Mefford et al. 2019).

A further category are genetic variants, which are rare or very rare, but have large effects in carriers. Known examples are the genes for FH (Utermann et al. 1989; Van der Hoek et al. 1997; Kraft et al. 2000), abetalipoproteinemia (Menzel et al. 1990), lipoprotein lipase deficiency (Sandholzer et al. 1992a), and LCAT deficiency (Steyrer et al. 1994).

Lp(a), CHD, and Mendelian Randomization

The role of Lp(a) in cardiovascular disease has long been debated and the debate followed an up and down parkour. The very first study reporting an association observed a higher frequency of “Lp+” among patients with myocardial infarction compared to controls (Renninger et al. 1965). This study of poor quality was published in German language and largely ignored. The field started with the publications of Dahlen in Sweden (1974), who reported an association of “pre-beta1-lipoprotein/Lp(a)” with CHD (Frick et al. 1974; Berg et al. 1974; Dahlén et al. 1975). In a highly cited paper, Gerhard Kostner and colleagues reported increased Lp(a) levels in patients with CHD over controls and defined 30 mg/dL as the threshold for elevated Lp(a) in plasma (Kostner et al. 1981), a value which was used in practice until recently. Histological demonstration and quantification of Lp(a)/apo(a) in the aortic wall and atherosclerotic plaques strengthened the idea that Lp(a) is a risk factor for cardiovascular disease (Költringer and Jürgens 1985; Rath et al. 1989; Niendorf et al. 1990; Beisiegel et al. 1990). Further strong support evolved from the homology of apo(a) with plasminogen (McLean et al. 1987) and the functional studies based on this finding which assigned a dual role in the pathogenesis of cardiovascular disease to Lp(a). As a particle composed of LDL and apo(a), it was believed to be atherothrombotic (Loscalzo 1990). At the beginning of the 1990th, the view of most researchers in the field was that Lp(a) is a risk factor which was summarized in a popular paper by Richard Lawn in “Scientific American” (Lawn 1992) with the title “*Lipoprotein(a) in Heart Disease.*”

Until then, all epidemiological studies relating Lp(a) to coronary risk were retrospective case–control studies. Circumstantial evidence for the pathogenicity of Lp(a) was in addition deduced from functional studies and histology. To gain further

insights into the metabolism, function, and pathophysiology of Lp(a), research groups started to generate transgenic mice first for apo(a) (Lawn et al. 1992) followed by double transgenics for apo(a) and LDL (Linton et al. 1993; Callow et al. 1994, 1995). These studies ended with an unresolved controversy. The double transgenics indeed had Lp(a) in plasma. Results on the development of atherosclerosis in these animals were however controversial. Atherosclerotic plaques were reported for transgenics expressing apo(a), suggesting that apo(a) unbound to LDL is atherogenic (Lawn et al. 1992). The same group reported that plaque formation is significantly (eight-fold) increased in apo(a)/human apo B double transgenics (Callow et al. 1995). These results were not confirmed by another study: neither mice expressing apo(a) alone nor double transgenics for apo(a) and human apoB developed significant aortic fatty lesions (Mancini et al. 1995a). Taken together, these animal models did not provide additional strong evidence that Lp(a) is a risk factor for atherothrombotic disease.

Studies in humans had the potential to change this when the concept of Mendelian randomization was applied in human epidemiologic studies—though the term had not been coined at the time. Numerous association studies starting end of the 1970th had investigated the relation between genetic polymorphisms and lipid levels, apolipoproteins, or the sequelae of atherothrombotic disease. The effect of the apoE polymorphism on lipid, lipoproteins, hyperlipidemia, and CHD was the first of this kind (Utermann et al. 1977, 1979, 1984; Menzel et al. 1983). These studies were not performed to answer the question whether the respective intermediate was likely a causal factor in the pathogenesis of the disease and did not follow the principle of Mendelian randomization. The questions were rather whether genetic variation contributes to the variation of the intermediate, e.g., LDL-C and if so what the mechanism might be. The observation that high LDL levels or low HDL levels were risk factors for CHD was accepted knowledge at the time (Humphries et al. 1992; Paulweber et al. 1988). Another question of these association studies was whether apolipoprotein genetic variation could be used as predictive markers for CHD (Hegele et al. 1986; Hegele and Breslow 1987). Katan had first formulated the principle of Mendelian randomization in a letter in *Lancet* (Katan 1986) following discussions with Gerd Assmann and the author of this article at a European Lipoprotein Club meeting. At the meeting, Katan had reported on an epidemiological study showing that patients with ovarian cancer had low plasma cholesterol. The question what was first, the hen or the egg, was unanswered and it was suggested to Katan to determine apoE isoforms in the patients and controls. Given the effect of the apoE polymorphism on cholesterol levels, this should result in differences in apoE allele frequencies between the groups in case that low cholesterol is causal for the disease. This discussion marked the birth of the concept of Mendelian randomization studies (Katan 2004; Davey Smith and Ebrahim 2004) (Fig. 1.8).

The very first study which applied a Mendelian randomization approach investigated the possible contribution of Lp(a) to coronary risk in patients with familial hypercholesterolemia (FH) using apo(a) isoforms for stratification (Seed et al. 1990). In this study, the principle was applied but not clearly defined. This was followed by two studies by Christoph Sandholzer and colleagues from Innsbruck in

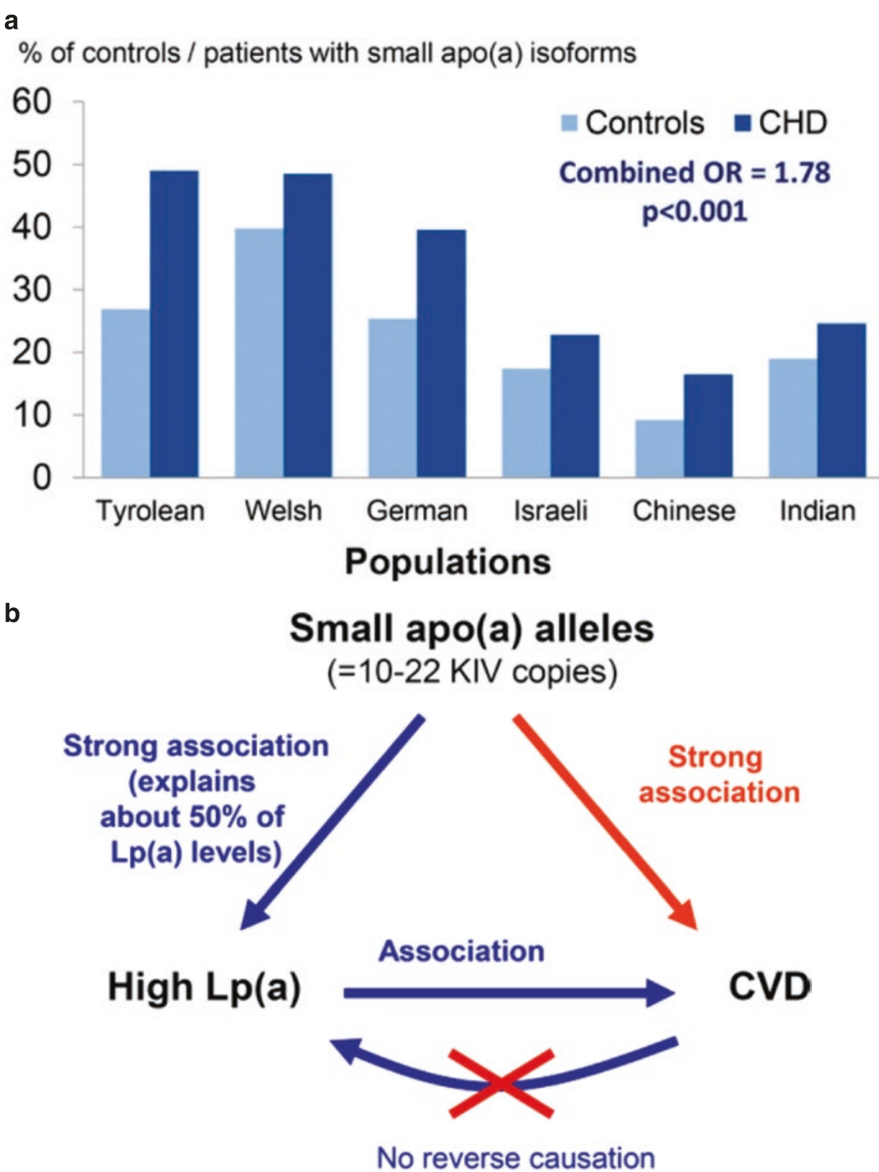

Fig. 1.8 Panel (**a**) Histogram showing the distribution of binned short and long apo(a) isoform frequencies in patients with CHD and controls in six populations (Data from Sandholzer et al. 1992c converted into graphic form. Adopted from Schmidt et al. 2016 with permission). Panel (**b**) Schematic illustration of the principle of Mendelian Randomization as first applied for Lp(a) in the studies of Sandholzer et al. (1992b, c). (From Kronenberg and Utermann 2013, used with permission)

which the principle of Mendelian randomization was clearly described. To cite from these papers: "*This is the first study which firmly establish a relationship between genetic apo(a) isoforms, Lp(a) levels and CHD*" and "*The data demonstrate that alleles at the apo(a) locus determine the risk for CHD through their effects on Lp(a) levels and firmly establish the role of Lp(a) as a primary genetic risk factor*" (Sandholzer et al. 1992b).

For the time these studies were performed, they were large including 355 CHD patients and 399 controls from China (Sandholzer et al. 1992b). The second study (Sandholzer et al. 1992c) was even larger with more than 1.000 patients and controls from six ethnic groups in which Lp(a) concentrations and apo(a) isoforms were determined. In both studies, small isoforms (i.e., isoforms with fewer KIV-2 repeats) which determine higher Lp(a) levels were significantly more frequent in CHD patients than in controls (Sandholzer et al. 1992c) (Fig. 1.8). In a further smaller study, apo(a) KIV-2 genotypes were determined by PFGE/Southern blotting together with apo(a) isoforms, Lp(a) levels, and disease status in patients that had undergone coronary angiography. The results confirmed that apo(a) alleles with low KIV-2 copy number and high associated Lp(a) concentration were significantly overrepresented in the patients (Kraft et al. 1996a). Despite the small sample size, the highly significant results reflected the fact that genotypes and expression level of each allele was known.

These studies apparently were premature and at odds with some prospective studies, which were the gold standard at the time. Though a first small prospective study by Rosengren et al. (1990) reported serum Lp(a) as independent risk factor for myocardial infarction in middle aged Swedish men, two subsequent studies, one from Finland (Jauhiainen et al. 1991) and a large study from the US (Ridker et al. 1993), failed to find significant associations and concluded that Lp(a) is not an independent risk factor. This provoked editorials in leading medical journals titled "*Has Lipoprotein 'little'(a) Shrunk?*" (Barnathan 1993). Subsequent prospective studies (Schaefer et al. 1994; Cremer et al. 1994) and meta-analysis of a large number of prospective studies published over the following years showed that these studies were clearly outliers and found a strong association of Lp(a) concentration with myocardial infarction and related phenotypes (Danesh et al. 2000; Bennet et al. 2008; Erqou et al. 2009). Today, it is known that the large influential study by Ridker et al. (1993) was flawed by problems with Lp(a) quantification which was clarified by the group in a later less prominently published paper (Suk et al. 2006). The approach to relate apo(a) isoforms to CHD was also taken up by several groups and meta-analysis of a series of 40 studies including 58.000 participants confirmed the seminal studies of Sandholzer et al. (1992b, c) on the association of isoform size with CHD (Erqou et al. 2010). Even this did not convince the entire community.

Only by the large Mendelian randomization studies of groups in Copenhagen/Denmark (Kamstrup et al. 2009) and Oxford (Clarke et al. 2009) Lp(a) was finally "*…resurrected by genetics*" (Kronenberg and Utermann 2013). Borge Nordestgaard's group determined Lp(a) levels and the sum of KIV-type-2 repeats from both apo(a) alleles by quantitative PCR (qPCR) in relation to CHD in participants from the

Copenhagen City Heart Study. They demonstrated a strong relation between Lp(a) levels, repeat number, and disease risk which was highest for individuals with high Lp(a) concentration and low sum of repeat numbers (Kamstrup et al. 2009). The conclusion from this study: "*These data are consistent with a causal association between elevated Lp(a) levels and increased risk of MI*" (Kamstrup et al. 2009) was almost identical with the one from the early isoform studies (Sandholzer et al. 1992b,c). Resurrection had happened twice but unlike in the bible, one was not enough. The second resurrection again changed headlines in journals, e.g., in "*Lipoprotein(a): There's life in the old dog yet*" (Kronenberg 2014a) or "*Lipoprotein(a): the underestimated cardiovascular risk factor*" (Thompson and Seed 2014) and finally triggered the development of drugs to lower Lp(a) in people with increased risk.

If genetically elevated Lp(a) levels increase risk for CVD as shown, genetically lowered Lp(a) should result in the opposite, i.e., risk reduction. This was in fact shown in population-based Mendelian randomization study from Finland (Lim et al. 2014) by the PROCARDIS study in Germany (Kyriakou et al. 2014) and by Stefan Coassin, Florian Kronenberg, and colleagues in Innsbruck who tested this hypothesis in two large studies (Coassin et al. 2017; Schachtl-Riess et al. 2021): they discovered two common splice site variants (4925G>A and 4733G>A) newly detected by deep sequencing in the KIV-2 repeat which both decreased Lp(a) concentrations tremendously (Coassin et al. 2017; Schachtl-Riess et al. 2021). The 4925G>A variant is observed in about 22% of European populations and is associated with smaller isoforms (mainly 19–25 K-IV repeats) and decreases Lp(a) concentrations by roughly 30 mg/dL. Carriers of these smaller isoforms who carry at the same time the 4925G>A splice site variant have a decreased risk for CHD (Coassin et al. 2017) which has also been confirmed by an Icelandic study (Gudbjartsson et al. 2019). The other splice site variant 4733G>A is with 38% even more frequent and occurs over a wide apo(a) isoform range and lowers Lp(a) by 13.6 mg/dL and also the risk for CHD (Schachtl-Riess et al. 2021). Using data from more than 440.000 participants from the UK Biobank revealed that carriers of both variants have low Lp(a) concentrations and a 12% decreased risk for CHD compared to non-carriers of the two mutations (Schachtl-Riess et al. 2021).

Data from an Icelandic study confirmed that Lp(a) levels are associated in a dose-dependent manner with risk for CAD, PVD, aortic valve stenosis, heart failure, and lifespan (Gudbjartsson et al. 2019). Short apo(a) alleles were also associated with risk but no additional residual association beyond the association with Lp(a) levels was observed for the KIV-2 polymorphism when Lp(a) was at the same time in the statistical model (Gudbjartsson et al. 2019). This can be explained by the fact that the Lp(a) concentration is the measured biological exposure which is only explained partially by the K-IV repeat polymorphism or other genetic variants.

The Mendelian randomization approach was further used to estimate the magnitude of a drug effect on Lp(a) to achieve a desired reduction of the risk for CHD (Burgess et al. 2018; Lamina and Kronenberg 2019; Madsen et al. 2020).

Non-Genetic Effects, Renal Disease, and Type 2 Diabetes

Estimates of the magnitude of the effect of the *LPA* locus on Lp(a) levels may be misleading. The studies from which the estimates were derived were performed on random population samples and healthy sib–pairs and families. The high heritability of more than 90% does therefore not exclude that rare and common conditions, e.g., diseases not represented in the sample may have significant effects on Lp(a) in affected individuals and groups and add to their health problems. Early studies on the effects of environment and various disease states were small, definition, treatments, and subtypes of disease differed between studies, and with few exceptions, they were controversial.

It was early recognized that Lp(a) levels in an individual may not be stable over time. Hormones and particularly changes in hormone levels during puberty and pregnancy were recognized as a cause (reviewed in Kostner and Kostner 2004) but slight fluctuations without apparent reason seem to be normal. Nutrition and physical activity have no effects.

Of clinical relevance are two associations of Lp(a) beyond the one with CAD. One is the association with chronic kidney disease (CKD) and the other with type 2 diabetes mellitus (T2D). The relationship of Lp(a) with renal disease including CHD in CKD is particularly complex. The first observation on elevated Lp(a) in hemodialysis patients probably is by Papadopoulos et al. (1980) who described a high frequency of an additional "pre-beta-lipoprotein" band visible in agarose gel electrophoresis in the patients. This "pre-beta-lipoprotein" appears identical with the "pre-beta1-lipoprotein" described by Dahlen to be associated with CAD (Dahlen 1974; Frick et al. 1974) and identified as Lp(a) (Berg et al. 1974). H. Parra in Jean-Luis Fruchart's group in Lille/France first reported Lp(a) elevation in patients with chronic renal failure (Parra et al. 1987b). Numerous studies followed which confirmed the observation and further revealed that the extend of Lp(a) increase depends on the type and treatment and that the increase in Lp(a) may have different causes, i.e., impaired removal from the circulation (Frischmann et al. 2007) or increased synthesis (Kronenberg et al. 1996). The increase is higher in patients with end-stage renal disease treated by continuous ambulatory peritoneal dialysis compared to those under hemodialysis (Kronenberg et al. 1996; Kronenberg 2014b). Patients with nephrotic syndrome develop excessive elevations of Lp(a) (Kronenberg et al. 1996, 2004; Takegoshi et al. 1991; Wanner et al. 1993). Following renal transplantation Lp(a) levels decrease to almost normal concentrations (Kronenberg et al. 1994a) demonstrating that the increased Lp(a) levels are secondary to disease (Kronenberg et al. 1994a; Black and Wilcken 1992).

Patients with ESRD have an increased risk for arterial vascular disease. Therefore, the report of Cressman and colleagues (1992) describing elevated Lp(a) as independent risk factor for CHD was noted with interest and triggered several follow-up studies. Though confirmed by most studies, it turned out that it was not the full truth but matters were more complicated. Apo(a) isoforms also play a role as demonstrated by Florian Kronenberg, Hans Dieplinger, and colleagues in Innsbruck who

unrevealed the complex interplay between renal disease, Lp(a) levels, and atherosclerotic vascular disease (Kronenberg et al. 1994b, 1999a, b; Koch et al. 1997). Despite higher Lp(a) levels in the patients, isoform frequencies were not different from controls (Dieplinger et al. 1993; Kronenberg et al. 1995). The pronounced increases in levels in patients were associated mainly with the longer isoforms and much less so with the short isoforms (Kronenberg et al. 1995, 1996; Dieplinger et al. 1993). The extend of Lp(a) increase with renal disease is mirrored by the rapid reduction following renal transplantation which is also dependent on the genetic phenotype (Kronenberg et al. 1994a). In heterozygous patients with one short and one long isoform which were analyzed before and following transplantation the change in concentration of the long, but not short isoform was impressively demonstrated by Western blotting (Kronenberg et al. 2003).

As in the general population, apo(a) phenotypes predicted the risk for atherosclerotic vascular disease in ESDR patients (Kronenberg et al. 1994b, 1999a) but surprisingly and in contrast to the work of Cressman and colleagues (1992) Lp(a) levels were found to be poor predictors. This paradoxical situation was explained by the different timelines of events. The increase of Lp(a) in patients with high molecular weight phenotypes starts only with the onset of disease and therefore does not last long enough for a significant pathogenic effect. In contrast, the exposure to high Lp(a) is lifelong in patients with low molecular phenotypes resulting in more preinjury and rapid development of atherothrombotic vascular disease. Lp(a) levels lose their predictive power whereas apo(a) types retain it. Kronenberg coined the term "galloping" atherosclerosis for the rapidly progressive form of vascular disease in ESDR patients with small apo(a) isoforms (Kronenberg et al. 1994b). As for most disease associations in the Lp(a) field, the reported association of apo(a) isoforms with ASVD in CKD patients was not confirmed by all and is controversial. In a recent review (Hopewell et al. 2018), it was concluded that CKD patients with high Lp(a) levels are at increased risk whereas it is unclear whether apo(a) isoforms are predictive for ASVD.

The situation on the role of Lp(a) in T2D is far from clear and illustrates how in some areas of Lp(a) research there was little progress over longer periods. Early small case-control studies from the 1990s were controversial. Some reported elevated (Bruckert et al. 1990; Heller et al. 1993), some lower (Rainwater et al. 1994), and some no change in Lp(a) levels (Császár et al. 1993) compared to controls. Rainwater et al. first reported decreased Lp(a) levels and larger apo(a) isoforms (Rainwater et al. 1994). Prospective studies investigating the role of Lp(a) as risk factor for incident or prevalent T2D observed an association of very low Lp(a) levels with disease (Mora et al. 2010; Ye et al. 2014; Paige et al. 2017) but it was unclear whether low Lp(a) is causally related to disease. To clarify this several groups initiated Mendelian randomization studies. These were, however, controversial. In a Mendelian randomization study from Copenhagen (Kamstrup and Nordestgaard 2013), no causal relation of low Lp(a) with T2D was observed and the role of long KIV-2 repeats remained unclear. A study in Chinese patients with CHD in which Lp(a) levels and KIV-2 repeats were determined, both low Lp(a) and high repeat number were associated with T2D. This resulted in the conclusion that low Lp(a)

predisposes to T2D (Mu-Han-Ha-Li et al. 2018). This view was challenged by the work of Tolbus et al. (2017) who used SNPs tagging Lp(a) levels or KIV-2 repeat number. They found high KIV-2 repeat numbers but not Lp(a) levels associated with T2D. In the very large population-based Iceland study, Kari Steffansson's group (Gudbjartsson et al. 2019) analyzed the association of loss-of-function (LOF) mutations in the *LPA* gene for a Mendelian randomization approach. These included known "null" mutations in *LPA* which are associated with very low or no Lp(a) in plasma. Presence of these mutations increased the risk for T2D in the Icelandic population. This to date is the most convincing evidence that very low Lp(a) predisposes to T2D. The mechanism underlying this relation is unknown but if known could shed new light on the still unresolved question for what reason evolution created Lp(a).

The Road to Therapy

The recommendations for individuals with a high risk for CHD or which suffered already from MI, angina pectoris, and related phenotypes and had elevated Lp(a) in plasma was for a long time to reduce other risk factors more rigorously. There were simply no drugs available. Niacin was the first recognized to lower Lp(a), but the effect was small the drug affected also LDL and HDL metabolism and had undesired side effects. An unexpected disappointment was that HMG-CoA-reductase inhibitors which effectively lower LDL by increasing LDL receptors at the cell surface did not lower Lp(a) (Kostner et al. 1989). The next surprise was that PCSK9 inhibitors, which also exert their effect on LDL by increasing LDL receptor-mediated uptake of LDL did also decrease Lp(a) concentrations (Raal et al. 2014) and are particularly effective in patients with familial hypercholesterolemia and elevated Lp(a) (Vuorio et al. 2020). The Lp(a)-lowering effect of PCSK9 inhibitors was shown in cell culture experiments to be mediated through an overexpression of LDL receptors and increased internalization of Lp(a) by the receptors (Romagnuolo et al. 2015). This is at odds with the lack of an effect of HMG-CoA-reductase inhibitors and presently unexplained. PCSK9 inhibitors are now a choice for treatment of at-risk patients with high Lp(a), but do not selectively lower Lp(a) in patients with very high Lp(a). Only recently, Lp(a) has been specifically targeted by antisense therapy against apo(a) (Tsimikas et al. 2015; Graham et al. 2016) with ongoing phase-III trials. A method, which has been very effective in reducing the risk for MACE in patients with severe clinical CHD, is lipoprotein apheresis (Jaeger et al. 2009; Roeseler et al. 2016; Pottle et al. 2019; Schettler et al. 2017). This therapy had been developed by the groups of Walter Stoffel in Cologne, which used antibody columns (Stoffel et al. 1981) and Dietrich Seidel in Göttingen who used heparin linked to columns (Eisenhauer et al. 1987; Armstrong et al. 1989) to absorb LDL and Lp(a) from plasma. This therapy is presently still the only one with a proven significant risk reduction in patients with severe CHD.

Outlook

A historical article describes the development of a scientific field from the past until the present day but not beyond. But can we learn from history for the future? What we certainly can learn is where the gaps in our knowledge are and what the important questions for the future might be.

In Lp(a) research, one major open question is what the physiological function of Lp(a) may be, a question which to the authors mind is tightly linked to evolutionary genetic aspects. The rhetorical question of Brown and Goldstein "does evolution make sense" in their News and Views article in Nature accompanying the cloning and sequencing of the apo(a) cDNA (Brown and Goldstein 1987) may well be extended to Lp(a).

It has been concluded that Lp(a) has no significant function since a large number of Europeans is heterozygous or compound homozygous for "null mutations" in the LPA gene and consequently Lp(a) is absent in plasma; these individuals are healthy and without any signs of disease (Schmidt et al. 2006). This conclusion is not warranted. First, the conclusion can only be that Lp(a) has no health-related function under present day conditions in the respective population (i.e., Finns). Second and more importantly, it seems highly unlikely that a macromolecular complex the assembly of which requires specific sites for non-covalent interaction between two extremely different large components, a lipoprotein and a glycoprotein, and the formation of a covalent disulfide bridge between them being present in all Old-world monkeys has survived for 40 Mio years when it has no function.

Considering that Lp(a) concentrations on average are higher in South-East Asians and severalfold higher in Africans compared to Europeans and that "null mutations" are significantly less frequent in Africans though Africans had longer time to accumulate deleterious mutations in none-essential genes, it may be speculated that Lp(a) has a significant role in Africa and further Old-world tropical populations. If Lp(a) has an unknown health-related function, e.g., in Africans, lowering Lp(a) by drugs for prevention of coronary disease may have undesired side effects. Large-scale studies of the Lp(a)/apo(a) trait in relation to disease, e.g., infectious disease in African populations may therefore elucidate the physiological role of Lp(a) and also have practical consequences for Lp(a) lowering drug regimes in these populations.

Acknowledgments I thank Anita Neuner for help with the literature, Eugen Preuss for preparing figures, and Florian Kronenberg for his support and for critically reading the manuscript.

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Chapter 2
Lp(a) Biochemistry, Composition, and Structure

Gerhard M. Kostner

Abbreviations

Apo(a)	Specific antigen of Lp(a)
ASGPR	Asialo-glycoprotein receptor
LCAT	Lecithin:cholesterol acyl transferase
LDL	Low-density lipoprotein
Lp	Lipoprotein
Lp(a)	Lipoprotein(a)

Historical Developments

In the early days, atherosclerosis research was dominated among others by two patho-mechanisms, one related to lipids and lipoproteins and the other to hemostasis and fibrinolysis. At that time, no one knew that Lp(a) constitutes a connection between them. Since lipids are mostly water insoluble, they have to be transported in blood complexed with amphipathic compounds such as phospholipids and apo-lipoproteins. The qualitative and quantitative separation of lipoproteins (Lp) was performed by (1) electrophoresis, (2) ultracentrifugation, and (3) by immune-affinity methods such as ELISA or immune-specific adsorbers. The nomenclature of lipoproteins reflected the separation methods and there were basically three classification systems: (1) based on the electrophoretic mobility yielding alpha-, ß-, and pre-ß Lp; (2) density classes with the main fractions VLDL, LDL, and HDL; and (3) Lp families with the main fractions Lp-A, Lp-B, and Lp-C (reviewed in Kostner 1983).

G. M. Kostner (✉)
Institute of Molecular Biology and Biochemistry, Medical University of Graz, Graz, Austria
e-mail: Gerhard.kostner@medunigraz.at

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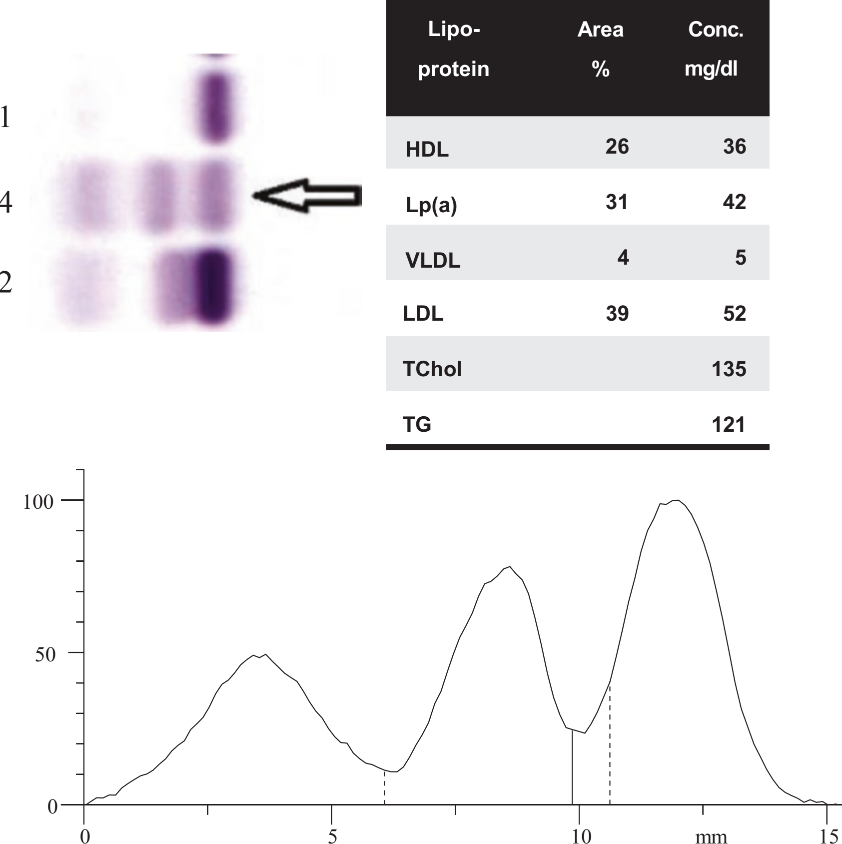
K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_2

The nomenclature relating to electrophoretic separation was used by the "East-Coast Lipid Laboratories" represented by Don Fredrickson and his collaborators (1967), the density fractions were propagated by John Gofman and Frank Lindgren from the "West-Coast group at the Donner Laboratories" (Gofman et al. 1949), and the ABC concept was pushed by Petar Alaupovic from Oklahoma City (Alaupovic et al. 1972).

The major component of VLDL and LDL, apo-LpB was recognized as a rather polymorphic apo-Lp with numerous allotypes and polymorphic form, that were described in several publications by Allison and Blumberg (1961). Allison and Blumberg tested sera from multi-transfused patients by immune diffusion (Ouchterlony test) for the presence of iso-antibodies against LDL. Many of these antibodies turned out to be unique, as they showed no cross-reactivity in Ouchterlony tests. The different polymorphic forms of apoB detected in this exercise were classified in the "Ag-system" where Ag stands for "antigen." The iso-precipitins described by Allison and Blumberg were not readily accessible by the rest of the scientific community and in order to be independent from the laboratory of Allison and Blumberg, Kare Berg, a geneticist from the university of Oslo, Norway, took another line by using xeno-antibodies against apoB (Berg 1963). He set out to hyper-immunize rabbits with LDL isolated from 20 healthy arbitrarily chosen donors. Although these antisera could not distinguish between the LDL from the donor individuals, K. Berg cross-absorbed the antisera with individual LDLs and finally came up with an immune serum that recognized an unique "Lp antigen" that he called Lpa. Lpa was present in some, but not in all sera testes from his patients. Sera that were positive for this factor were called Lpa+ and those negative were called Lpa−. In a panel of 314 sera from healthy adult donors, 34% were Lpa+ and the remaining ones Lpa-negative. Notably, in our own studies, we quantified Lp(a) in a group of 107 healthy and 76 myocardial infarction (MI) patients from Venice and found that 35% of them had Lp(a) levels of >30 mg/dL, the value that had been adopted as the cut-off for coronary heart disease (CHD) and MI in numerous subsequent studies (Kostner et al. 1981). In early days, Lp(a) was considered to be a qualitative genetic trait and a gene frequency of 0.1881 was calculated in the Norwegian population (Berg 1963). In the Ag system mentioned above, some 14 different alleles were characterized that obviously reflected some sequence variations in the *APOB* gene or possibly variations in the sugar moiety (reviewed in Kostner 1976). Additional independent polymorphisms of apoB that might not be related to the Ag system, called the Tl system (from "trypsin-treated Lps") and the El system (from "electrophoresis"), have been described, but did not get much attention in the following years (reviewed in Kostner 1976). Other suggested polymorphisms, the Ld system and the Lt system, turned out to be in fact Ag alleles (Utermann 1989). For completeness, it is noteworthy to mention "Lp(x)" that was described by Bundschuh and Vogt (1965) as a factor distinct from Lp(a). Lp(x) was identified with xeno-antibodies from horse, but not from rabbits, and was believed it to be a heterologous form of Lp(a). All these polymorphisms were more or less forgotten in later years, as they apparently had no relevance for atherosclerosis or cardiovascular diseases.

The current view of the buildup of Lp(a) emerged from several subsequent studies carried out in the laboratories of A. Scanu (Fless et al. 1985), our own, and several others. In fact, it is mostly believed that there exists in human plasma one rather homogenous fraction of Lp(a) consisting of a bona fide LDL and one apo(a) glycoprotein covalently linked by a disulfide bridge. Whether or not this reflects the true *in vivo* situation under all circumstances remains to be established.



Lipoprotein	Area %	Conc. mg/dl
HDL	26	36
Lp(a)	31	42
VLDL	4	5
LDL	39	52
TChol		135
TG		121

Fig. 2.1 Separation of Lp(a) by electrophoresis: Lp(a) migrates in gel electrophoresis as extra pre-ß1 band and may be quantitated either after staining for lipids with Sudan black, or by staining with a cholesterol reagent. Here, Lp(a) is separated by the Helena® Electrophoretic system https://www.helena.com/. The concentration in mg/dL refers to Lp(a)-cholesterol as staining was performed with a cholesterol dye. Since Lp(a) consists of some 25–30% of cholesterol, Lp(a) mass in mg/dL may be calculated by multiplication with a factor of 3–4. (1 and 2): Plasma with Lp(a) of <30 mg/dL; (4) plasma with a Lp(a) concentration of 140 mg/dL. (From: Kostner, K.M.; Kostner, G.M. Lp(a) and the Risk for Cardiovascular Disease: Focus on the Lp(a) Paradox in Diabetes Mellitus. Int. J. Mol. Sci. **2022**, 23, 3584. https://doi.org/10.3390/ijms23073584)

Purification and Composition of Lp(a)

In the first reports by K. Berg and his collaborators, Lp(a) was denominated "pre-ß1" and/or "sinking pre-ß lipoprotein" (Berg 1963). In paper or agarose gel electrophoresis, Lp(a) migrated somewhat faster than pre-ß-Lp (VLDL) but slower than alpha-Lp (HDL). This is depicted in Fig. 2.1. It must, however, be mentioned at this point that the actual position of Lp(a) by electrophoretic methods depends on the carrier material, the type, and pH of the electrophoresis buffer and the presence of anti-coagulants such as heparin.

The purification of Lp(a) succeeds by a combination of methods including ultracentrifugation, poly-anion precipitation, size exclusion chromatography, and affinity chromatography. In the case of density gradient ultracentrifugation, most of the Lp(a) from fasting plasma of healthy individuals is found between LDL and HDL_2 as shown in Fig. 2.2. These properties of Lp(a) in electrophoresis and ultracentrifugation led to its term “sinking pre-ß lipoprotein” in early publications.

As will be detailed later, apo(a) is characterized by a unique size polymorphism with great differences in their molecular mass. It is therefore evident that different isoforms of apo(a) cause significant different hydrated densities of the corresponding Lp(a) that may be found at variable positions in the density gradient. In the case of heterozygous individuals with striking differences of the apo(a) mass, even two distinct Lp(a) bands may be found in the density gradient.

It must be emphasized here that apo(a) is not only found in a single distinct fraction by ultracentrifugation, but there is rather a distribution over the whole density gradient in most of the plasma samples of blood donors. This has been emphasized particularly in a review article published by Fless (1990).

In the plasma sample shown in Fig. 2.2, only some 75% of apo(a) was found between LDL and HDL, and there were appreciable amounts also found in the VLDL, HDL, and bottom fraction. If one separates post-prandial plasma or plasma from hypertriglyceridemic patients by density gradient ultracentrifugation, the

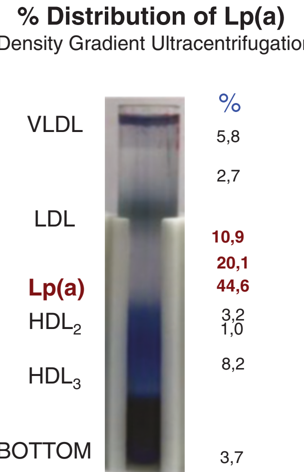


Fig. 2.2 Separation of Lp(a) by density gradient ultracentrifugation that highlights the heterogenous nature of Lp(a). After prestaining all serum constituents with Coomassie blue, lipoproteins were separated by ultracentrifugation in the SW-41 Rotor, Beckmann® for 24 h at 40,000 rpm. In the particular plasma, some 75% of Lp(a) was found in the HDL_1 region, the rest distributed between the top up to the bottom fraction. (From: Kostner, K.M.; Kostner, G.M. Lp(a) and the Risk for Cardiovascular Disease: Focus on the Lp(a) Paradox in Diabetes Mellitus. Int. J. Mol. Sci. **2022**, 23, 3584. https://doi.org/10.3390/ijms23073584)

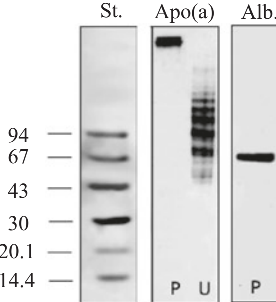


Fig. 2.3 Western blot of Apo(a) isolated from plasma or urine. Plasma Lp(a) was purified as described in Kostner et al. (1999) from a donor containing 90 mg/dL of Lp(a). The urine of the same individual was concentrated 50-fold and both fractions were separated by SDS–polyacrylamide gel electrophoresis followed by W-blotting. St: protein molecular weight standard, the numbers indicating the mass in kDa. P refers to plasma and U refers to urine. Alb: Plasma albumin used as a reference. (From: Kostner, K.M.; Kostner, G.M. Lp(a) and the Risk for Cardiovascular Disease: Focus on the Lp(a) Paradox in Diabetes Mellitus. Int. J. Mol. Sci. **2022**, 23, 3584. https://doi.org/10.3390/ijms23073584)

situation is even more complex and much larger amounts of apo(a) are found in VLDL or IDL. The exact morphology of Lp(a) outside of HDL_1 has not been elucidated in detail. Whether they are artifacts or true metabolic entities is not fully clear. We characterized the apo(a) immune reactivity found in the bottom fraction of human plasma and found that they consist of fragments created by Ca^{2+}-dependent proteases that are abundant on cell surfaces from several organs (Frank et al. 2001; Gries et al. 1987). These fragments are not bound to lipoproteins and have masses of some 50–150 kDa. Similar fragments of apo(a) are found in urine despite their rather large size (Kostner et al. 2001) (Fig. 2.3). The amount of apo(a) fragments in urine correlates significantly with the parent Lp(a) plasma concentration and we therefore proposed the use of urinary apo(a) as a clinical chemical risk parameter for atherosclerotic diseases (Kostner et al. 1996).

Preparation of Pure Lp(a)

The preparation of pure Lp(a) with high yield from plasma with low concentration—mostly corresponding to large apo(a) isoforms is not an easy task. Therefore, most investigators use plasma from donors with high Lp(a) values, that usually contain small apo(a) isoforms. These Lp(a) specimens have a density not much different from that of LDL and therefore by ultracentrifugation used mostly as a first step, large amounts of Lp(a) may be contaminated with LDL or may be lost in the LDL fraction. There are two more hassles that must be considered by purification of Lp(a) from various donors: (1) Lp(a) with large apo(a) isoforms in pure form are prone to spontaneous precipitation and (2) Lp(a) associates with numerous other

apo-Lp such as for example apoE, apoH (ß2-glycoprotein), and many kinds of other serum constituents. These Lp(a) complexes may be dissociated by the addition of amino acids such as Lys, Pro, hydroxy-Pro, and others. We therefore elaborated a purification procedure that yielded Lp(a) with a purity of some 98%. It was suggested that this material might be suitable for use as a "gold standard" in value assignment for clinical chemical analyses (Kostner et al. 1999).

In short, blood is harvested from donors with Lp(a) concentrations of >30 mg/dL and in the first step either citrate plasma or serum is prepared after coagulation. After adding some preservatives (EDTA, BHT, PMSF, thiomersal), all lipoproteins with $d < 1.060$ g/mL are separated by ultracentrifugation. Next, the density fraction >1.060 < 1.125 g/mL is obtained by ultracentrifugation. After concentration to approx. 10–20 mg/mL Lp(a)-cholesterol, proline at a final concentration of 0.1 mol/L is added. This step is essential as it dissociates all proteins from Lp(a) other than apo(a) and apoB. In the next step, lipoproteins are separated by size exclusion chromatography over Biogel A-15 m and the Lp(a) peak is harvested and concentrated by pressure dialysis. For storage over a longer time period, pure Lp(a) may be frozen at −20 to −70 °C in the presence of stabilizers. We tried several ones including saccharose, polyethylene glycol, and others and at the end found out that the 1:1 admixture of pure glycerol gave the best results. If prepared according to this procedure, Lp(a) shows one band in agarose gel electrophoresis and is also virtually pure by SDS–PAGE.

Chemical Composition of Purified Lp(a)

There is quite some variation in the composition of purified Lp(a) that is donor specific reflecting the isoform size, the lipid status of the plasma, and more. In Table 2.1., some average values are shown of Lp(a) isolated from fasting healthy donors in comparison with LDL.

It must be emphasized here that the composition of Lp(a) shown in Table 2.1. is just a snapshot of a fraction isolated from fasting plasma of normolipemic healthy individuals. Lp(a) outside of the HDL_1 density fraction or Lp(a) isolated from dys- or hyperlipemic plasma may have quite significantly deviant structures and compositions.

Table 2.1. Chemical composition of Lp(a) in comparison with LDL

Compound	Lp(a) % w/w	LDL % w/w
Protein	26–30	21.0
Carbohydrates	4–8	1.3
Cholesteryl ester	31–37	42.0
Free cholesterol	7–8	9.0
Phospholipids	16–20	20.7
Triglycerides	4–6	6.0

The Protein Structures of Lp(a) and Apo(a)

As mentioned above, the chemical composition of Lp(a) depends on the individual donor, its nutritional and health status, and the preparation procedure. An idealized Lp(a) particle separated from plasma at *d* 1.060–1.125 g/mL is composed of an LDL-like core lipoprotein with a lipid composition close to that of the LDL fraction of *d* 1.030–1.060 g/mL. To this core lipoprotein, the specific apo(a) glycoprotein is covalently linked by a disulfide bridge (Fig. 2.4). The disulfide bridge links Cys 4326 in apoB-100 with the only free Cys 4057 in apo(a), that is located in kringle four (K-IV) Type-9. Due to the size polymorphism, there is a great variation in the molecular mass of apo(a) that reaches from 350 to >800 kDa. There are only a few reports on the morphology and structure of Lp(a) revealed by physico-chemical methods and electron microscopy. An interesting view of Lp(a) has been published by Weisel et al. (2001) who studied Lp(a) architecture by rotary-shadowing electron microscopy. They proposed that the protein components of Lp(a) after exposure of glycerol consist of rings made up of dense nodules of various size. After exposure to tranexamic acid, apo(a) and apoB dissociated and apo(a) formed a long tail with distinct kringle units but still linked to the LDL core.

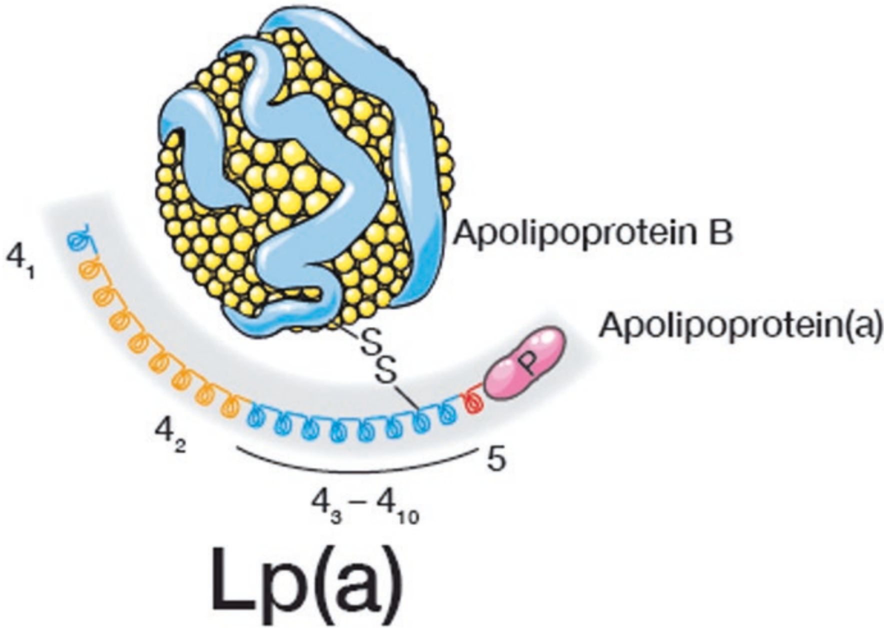


Fig. 2.4 Hypothetical model of Lp(a) showing the LDL core with apoB-100 as the major surface protein and apo(a). 4_1 refers to K-IVT-1, 4_2 to K-IV-T2, and so one. 5 = kringle V and P = the protease domain. (The figure was drawn by Timo Speer, Med. University of Homburg/Saar, Germany. From: Kostner, K.M.; Kostner, G.M. Lp(a) and the Risk for Cardiovascular Disease: Focus on the Lp(a) Paradox in Diabetes Mellitus.Int. J. Mol. Sci. **2022**, 23, 3584. https://doi.org/10.3390/ijms23073584)

The structure of apo(a), the characteristic glycoprotein component of Lp(a), has a unique structure as summarized by Morrisett in 1990 (Morrisett et al. 1990). It consists of repetitive protein segments, so-called kringles (K), that are highly homologous to K-IV in plasminogen. K-IV's contain approximately 110 amino acids forming a secondary structure, which resembles "Danish kringles" (McLean et al. 1987). The N-terminal part of apo(a) consists of various numbers of unique or repetitive copies of these kringle-IV's. In addition, apo(a) has one copy of a K-V like kringle and a non-functional protease-like domain, both highly homogenous to that of plasminogen. A cartoon of the apo(a) structure is shown in Fig. 2.5. In humans, there exist more than 30 genetic size polymorphisms of apo(a). The smallest apo(a) isoform consists of the protease domain, one copy of K-V and 11 K-IV's of which K-IV Type-1 (T-1) and T-(3–10) are unique in their primary structure, whereas K-IV T-2 is present in 2 identical copies. Larger isoforms differ by the number of K-IV T-2's; the largest apo(a)'s described so far had 52–54 K-IV's.

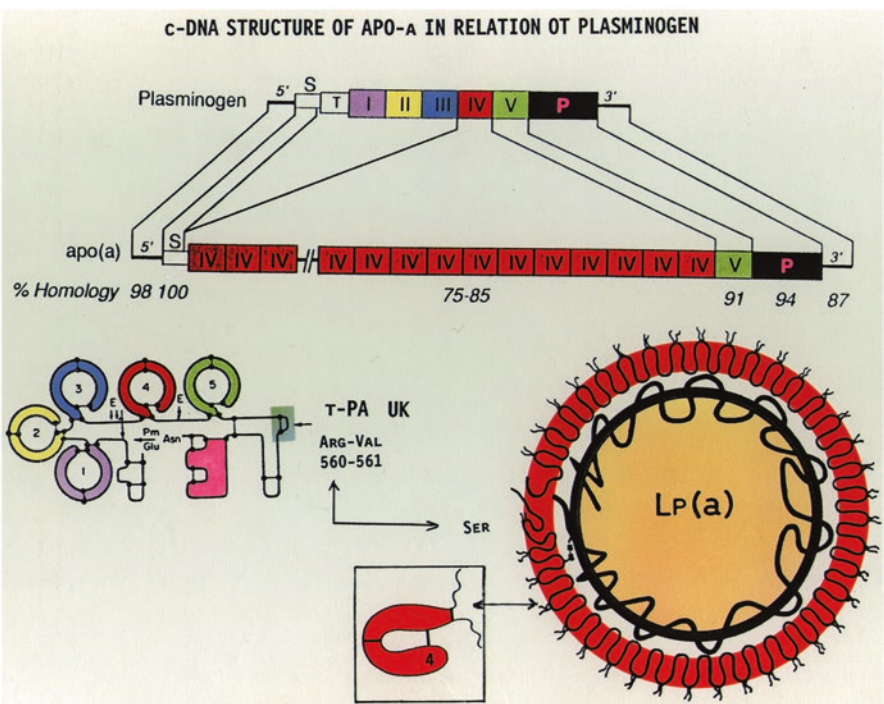


Fig. 2.5 cDNA structure of apo(a) in comparison to plasminogen. In apo(a), kringle-IV's (K-IV) homologous to plasminogen are repeated several times. There is also one K-V like domain and a protease domain in apo(a) (P) with a homology of 94 to that of plasminogen. The Arg of plasminogen is replaced in apo(a) by Ser and thus the protease cannot by activated in Lp(a) by t-PA or urokinase. (From: Kostner, K.M.; Kostner, G.M. Lp(a) and the Risk for Cardiovascular Disease: Focus on the Lp(a) Paradox in Diabetes Mellitus.Int. J. Mol. Sci. **2022**, 23, 3584. https://doi.org/10.3390/ijms23073584)

High-resolution structures of whole apo(a) have not been published, but there exist a few crystal structures of recombinant apo(a)-kringle-IV's (Ye et al. 2001). The crystal structure refined to a resolution of 1.45 Å revealed important structural features of kringle-IV-T-7 that are postulated to be responsible for the interaction with Lysine groups.

The Carbohydrate Moiety of Apo(a)

The K-IV domains are connected by linker regions that are highly glycosylated by N- and O-linked sugars. The best characterization of the apo(a) carbohydrate arrangement has been published by Garner et al. (2001) who demonstrated that approx. 20% of the oligosaccharide structures consist of two major Asp-linked N-oligosaccharides. N-glycans are complex biantennary structures in either a mono- or disialylated state. The remaining 80% of the sugars are Ser/Thr O-linked oligosaccharides and are present in all apo(a) isoforms. The majority consist of the mono-sialylated core type-I structure, NeuNAcα2-3Galβ1-3GalNAc, and the remaining consist of disialylated and non-sialylated O-glycans. The latter finding prompted us to elucidate the possibility that Lp(a) might bind to the asialo-glycoprotein receptor (ASGPR) on liver cells**.**

The Role of Structural Apo(a) Features for the Lp(a) Metabolism

It is well known that the sialic acid content of glycoproteins regulates their fate in circulating blood. Aged protein may be devoid of their terminal sialic acid due to the action of specific sialidases. There are specific asialo-glycoprotein receptors on liver cells that very effectively bind and catabolize such asialo-glycoproteins. This is schematically displayed in Fig. 2.6. ASGPR may be specific for galactose or mannose.

In a study we published in 2003 (Hrzenjak et al. 2003), it was important that antimicrobial agents, among other preservatives, were added immediately after drawing blood and also throughout the different isolation steps, in order to obtain "native Lp(a)" as far as possible. The metabolic experiments were carried out in hedgehogs, the only animal species with the exception of old-world monkeys that synthesize an Lp(a) like lipoprotein. In vivo experiments in these animals revealed that desialylated Lp(a) is catabolized 25 times faster than native Lp(a) and is almost exclusively taken up by the liver. Concomitant injection of asialo-Lp(a) with asialo-orosomucoide to hedgehogs reduced the half-life of asialo-Lp(a) to values observed for native Lp(a). Mannan, the competitive inhibitor for the mannose-specific ASGPR, had no effect. Similar results were observed in wild-type mice where desialylated Lp(a) is catabolized 50–100 times faster than sialylated Lp(a). Here also, only asialo-orosomucoide, but not mannan, acted as a competitive inhibitor.

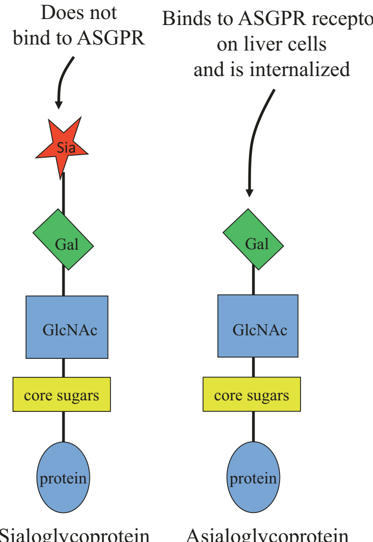


Fig. 2.6 The sugar moiety of glycoproteins consists of complex antennary structures, many of them containing sialic acid (Sia) as terminal sugar. Aged proteins may be targets of sialidases that cleave sialic acid off, and in turn, galactose (Gal) or mannoses may be exposed to the surface. These latter sugars are avidly bound to asialo-glycoprotein receptors (ASGPR) on liver cells and catabolized

Whether or not the results of these experiments might be transferable to the situation in humans cannot be answered from these experiments. Any Lp(a) preparation isolated from human plasma may contain only a small fraction of the non-sialylated Lp(a), because of its very short half-life in the circulating blood of the donor. Whether such putative non-sialylated Lp(a) is directly secreted from human liver or is generated during circulation in blood or might be an artifact generated during Lp(a) preparation remains to be investigated. It is, however, important to note that we also carried out similar metabolic studies with native Lp(a) in wild-type mice and in ASGPR knockout mice. In the ko-mice, the HL-2 subunit of the ASGPR was absent. When native freshly isolated Lp(a) was injected in either mice, we observed a measurable retardation of the Lp(a) catabolism in the KO-mice as compared to wild-type mice. The amount of Lp(a) labeled with the non-degradable isotope $^{[125I]}$tyramine cellobiose accumulating in the liver of knockout mice was significantly lower compared with wild-type mice (Hrzenjak et al. 2003). We believe this is a strong argument for the ASGPR pathway being indeed involved in Lp(a) catabolism in humans.

We also observed that some 87% of intravenously injected asialo-Lp(a) was cleared by ASGPR-negative mice within 1 h. Previous studies by Roos et al. (1983) demonstrated two galactose-specific receptors in rat liver: The Kupffer cell-specific glycoprotein receptor readily interacts with galactose-exposing particles of the size of LDL (Fadden et al. 2003). We assume that this receptor might be responsible for the clearance of asialo-Lp(a) in our ASGPR ko mouse model. Yet, the Kupffer cell-specific glycoprotein receptor is not expressed in humans, as human genome analysis reveals a pseudogene that is not translated into a protein (Van Berkel et al. 1985). Taken together, the role of the ASGPR for the catabolism of Lp(a) in humans is far from being clear and requires further investigation.

Impact of Gene Variants on the Apo(a) Structure

This topic is thoroughly covered in the article by G. Utermann in this book, and thus, I mention in this paragraph only a few aspects that might be relevant for structural considerations of Lp(a). Among the numerous polymorphisms and mutations described elsewhere in this book, there is in particular one mutation relevant here, the truncated form of apo(a) expressed by the so-called null allele. The first report on truncated apo(a) was published by M. Ogorelkova from the laboratory of G. Utermann in Innsbruck (Ogorelkova et al. 1999). Gene sequencing of *APOA* in Caucasian individuals with almost zero Lp(a) levels revealed a G→A substitution at the 1+ donor splice site of K-IV type 8 introns. This nonsense mutation led to the expression of a truncated form of apo(a) that consisted of a N-terminal fragment lacking all entities after kringle-IV-T7. Since K-IV T9 in apo(a) contains the single free –SH group that is necessary for the covalent binding to apoB-100, such truncated apo(a) are not able to stably assemble with LDL. Interestingly, there are small amounts of free truncated apo(a) found in plasma indicating that the liver secretes such apo(a) mutants, but it seems that they are very rapidly catabolized. This opens up the question whether plasma Lp(a) levels might be drastically reduced in general by inhibition of Lp(a) assembly.

Impact of the Assembly on Plasma Concentrations of Lp(a)

We addressed this question in in vivo and in vitro studies using tranexamic acid for the inhibition of Lp(a) assembly (Frank et al. 1999). In vivo studies were performed with single transgenic apo(a) mice or double transgenic apo(a):apoB mice both of them carrying the relevant human genes. The assembly of apo(a) in the test tube may be fully inhibited by Lys or analogs thereof such as delta-amino valeric d-AVA acid or tranexamic acid TXA, the latter being the strongest inhibitor that is also known to inhibit fibrinolysis by plasmin (displayed in a cartoon in Fig. 2.7). The mice were fed 150 mg/dL of d-AVA or TXA for 1–2 weeks and the plasma apo(a) and Lp(a) concentrations were followed over time. In the double transgenic Lp(a) mice, in contrast to what we expected, the concentration of Lp(a) rose after 1 week of feeding to almost twice the value observed in the absence of d-AVA or TNX feeding. This was a transient effect since after omitting the inhibitors from the chow, the Lp(a) concentration returned to the pretreatment values. We also revealed that the increase of plasma Lp(a) was fully accounted for by the presence of genuine Lp(a) and not by free apo(a). When single transgenic apo(a) mice were treated in a similar protocol apo(a) rose after 1 week by 57% and returned to pretreatment values as well. Similar results were obtained by d-AVA feeding, yet they were less pronounced. We then harvested the livers of mice treated with TNX or d-AVA and found that their concentration in the liver was significantly lower than without treatment.

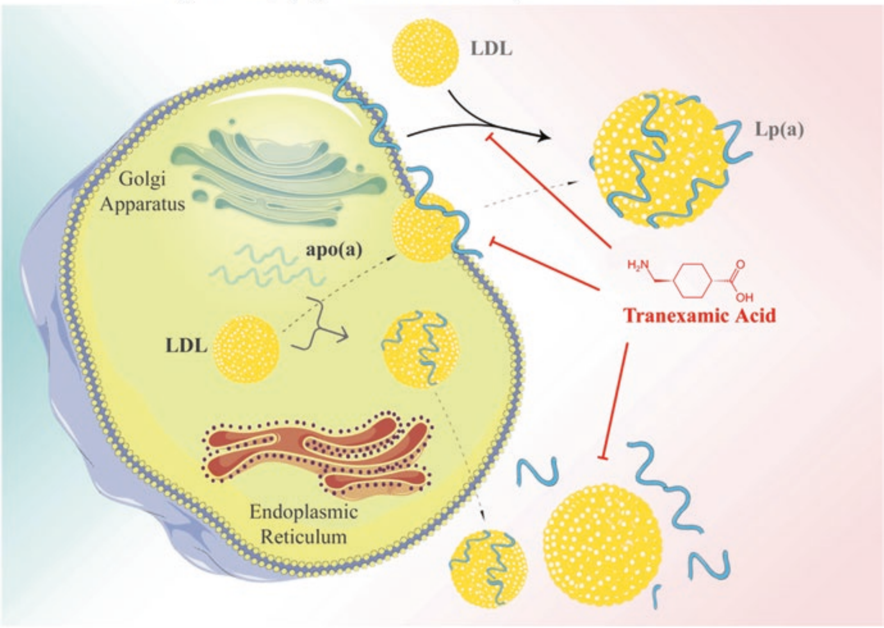


Fig. 2.7 Possible models of Lp(a) assembly and inhibition by tranexamic acid. There are currently two models of Lp(a) assembly discussed: (1) apo(a) is biosynthesized in the liver and after passage through the Golgi apparatus it binds to the surface of liver cells. Bypassing LDL associate with apo(a) and both components are covalently linked by a disulfide bridge. The first step of assembly, the interaction of kringles-4 with Lys groups of apoB-100 may be competed for by Lys analogs such as Tranexamic acid. Free apo(a) not complexed to LDL might be degraded by hydrolytic enzymes. Alternatively, the assembly may take place intracellularly in the liver cells. (From: Kostner, K.M. and Kostner, K.M. Lipoprotein(a): a historical appraisal. J Lipid Res. 2017, 58, 1–14. https://doi.org/10.1194/jlr.R071571)

In turnover studies carried out with TNX or d-AVA or without, we demonstrated that half-lives of Lp(a) were prolonged by approximately 33% in the former. Moreover, in vitro experiments with McA-RH 7777-XL rat liver cells stably transfected with apo(a) helped to get insight in the possible mechanism of our findings. Cells treated with TNX or d-AVA showed lower amounts of expressed apo(a) in cell extracts, yet the amount of apo(a) in the medium was significantly increased. Our findings are compatible with the interpretation that apo(a) after biosynthesis and cell excretion is bound to liver cell surfaces where Lys groups at the surface are essentially involved. Surface bound apo(a) might be catabolized more rapidly, whereas dissociation from the surface by Lys analogs shuttles apo(a) into the circulation or into the incubation medium where its concentration increases. We believe that these findings have a profound impact on the individual plasma levels of Lp(a) in humans: The stronger the binding of newly synthesized apo(a) on the cell surface is, the lower is the final Lp(a) concentration in circulation. This might be reflected by the fact, that large apo(a) isoforms with a greater number of K-IV's bind stronger

to liver cell surfaces and are partly degraded before assembling with LDL to intact circulating Lp(a).

Impact of Apo(a) Mutations on Coronary Artery Disease (CAD)

Given the fact that Lp(a) is a strong independent risk factor for atherosclerosis and CAD it was speculated that individuals with apo(a) mutations or polymorphisms that cause reduced plasma Lp(a) levels might be at a lower risk for CAD. This was addressed in a cohort of the PROCARDIS study published by Kyriakou (Kyriakou et al. 2014). Indeed, it was found that the LPA null allele as identified by the rs41272114 SNP not only is associated with reduced plasma Lp(a) concentrations but also with a significantly reduced CAD risk.

Among all the mutations in the whole apo(a) gene including the promoter region and regulatory cis-acting regions described in the literature, there are two publications from the Innsbruck laboratory that need attention (Noureen et al. 2015; Coassin et al. 2019). It was known since the first published report on the LPA gene sequence by McLean and Lawn (McLean et al. 1987) that there are silent mutations in the repetitive gene region coding for the K-IV T2. These variations were called K-IV$_2$ A and K-IV$_2$ B. Despite of the difference in the gene sequence, the A and B alleles translated into the same apo(a) protein sequence. Sequencing the apo(a) gene is not an easy task because of the repetitive structure caused by K-IV T2 and the homology of the non-repetitive kringles K-IV-1 and K-IV-3 to K-IV 10. Nevertheless, Stefan Coassin and his colleagues succeeded to elaborate a sophisticated protocol that allowed apo(a) sequencing in larger quantities. In these studies, gene variants in the K-IV-T2 region were identified that translated drastically into plasma Lp(a) concentrations. The exact mechanisms on a molecular level are not fully clear, but the results highlight the importance of the polymorphic *APOA* gene sequence for the apo(a) protein expression and plasma concentration of Lp(a).

Impact of the LDL Structure on the Lp(a) Assembly

The reason why apo(a) only assembles with LDL and not with other serum proteins that may have Lys groups exposed to the surface has never been fully explored. As a matter of fact, the greatest portion of full length apo(a)—if not all is found on apoB-100 containing lipoproteins in human plasma. This led us to assume that the composition and morphology of LDL have just the right prerequisites for this assembly. Two observations published earlier by our laboratory strongly support this concept.

Numerous reports in the literature demonstrate that Lp(a) hardly binds directly to the LDL receptor (Hofer et al. 1997). This is at a first consideration surprising, as some 50% of the Lp(a) moiety consist of apoB-100. The most plausible explanation that Lp(a) is not bound to the LDL-R would be that the large glycoprotein apo(a)

masks the epitope in apoB-100 responsible for LDL receptor binding. This concept is strongly supported by our studies of patients suffering from familial defective apoB-100 (FDB) (Durovic et al. 1994). FDB patients express a mutant apoB-100 where Arg at position 3500 is substituted by Gln. This substitution causes the biosynthesis of an LDL particle that has a strongly reduced binding affinity to the LDL receptor and in turn patients with FDB are hypercholesterolemic.

In the investigations published by Ernst Steyrer et al. (1994), we studied the in vitro assembly of Lp(a) by mixing purified LDL with recombinant apo(a) and followed the covalent linkage of both components. Whereas wild-type LDL mixed with apo(a) complexed under the given experimental conditions between 15% and 44% with apo(a), LDL from a homozygous FDB patient showed only 2–16% association. The corresponding figure using LDL from heterozygous FDB individuals was 2–30%. Moreover, we found that in heterozygous FDB patients the ratio of defective to wild-type apoB100 in Lp(a) is significantly lower than in LDL from the same patients. These results strongly suggest that the epitope in apoB-100 that is involved in LDL receptor binding is also highly relevant for apo(a) binding and covalent linkage.

Another example for the importance of the right morphology of LDL to warrant an ideal assembly has been published in 1994 by our group. It is known that patients suffering from LCAT deficiency (LCAT-D) have a grossly altered plasma lipoprotein pattern. We made also the observation that 9 heterozygous LCAT-D patients had only 2–13 mg/dL of Lp(a) whereas the Lp(a) concentration in the non-affected siblings was significantly higher (Ernst Steyrer et al. 1994). Eleven of the studied homozygous LCAT-D patients exhibited plasma Lp(a) levels of virtually zero. The morphology of lipoproteins in the LDL region isolated from homozygous LCAT-D patients was grossly altered with large vesicles and small spheres and there was an almost complete lack of cholesteryl esters (Fig. 2.8). When LCAT-D LDL were incubated with recombinant apo(a) for 20 h at 37 °C, no complex of

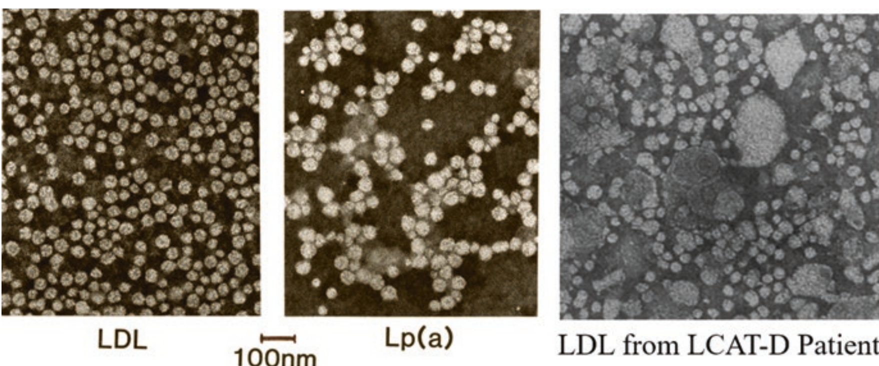


Fig. 2.8 Negative-stain electron microscopy of LDL (density 1.030–1.063 g/mL) and Lp(a) isolated from a healthy control individual and from a homozygous patient suffering from LCAT deficiency. (From: Kostner, K.M.; Kostner, G.M. Lp(a) and the Risk for Cardiovascular Disease: Focus on the Lp(a) Paradox in Diabetes Mellitus. Int. J. Mol. Sci. **2022**, 23, 3584. https://doi.org/10.3390/ijms23073584)

apoB100:r-apo(a) had been formed (i.e., no assembly to Lp(a) took place). Normal LDL under the same conditions showed complete assembly to Lp(a). We concluded that the integrity of the LDL structure is a prerequisite for the biosynthesis of genuine Lp(a). Furthermore, it appears that the lack of complexing apo(a) to bona fide LDL leads to a fast catabolism or degradation of the expressed apo(a). Thus, not only is an intact LCAT activity necessary, but there must also be an abundance of "normal" native LDL. These factors substantially regulate plasma Lp(a) metabolism and serum Lp(a) levels.

Disclosures I have nothing to disclose.

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Chapter 3
Genetics of Lipoprotein(a)

Gerd Utermann

Introduction

The genetics of lipoprotein(a) [Lp(a] is both, simple and complex at the same time. Initially described as a dominant trait (Berg 1963; Berg and Mohr 1963) with two immunologically defined phenotypes, Lp+ and Lp−, where Lp+ is dominant over Lp−, it is now well established that Lp(a) is a quantitative trait with a very broad distribution in all studied populations (Schmidt et al. 2016). Lp(a) concentrations vary more than 1000-fold between individuals in the same population and range from undetectable to >200 mg/dL in healthy individuals (Utermann 1989). In all populations, the distribution of Lp(a) levels is skewed and far from Gaussian. In Europeans, most individuals have low and few have very high Lp(a) concentrations. Mean and median Lp(a) concentrations, the distribution of Lp(a) concentrations (e.g., skewness) vary widely between human ethnic groups. Sub-Saharan Africans have by far the highest levels and lowest skewness. Compared to Europeans, they are two to fourfold higher. The highest concentrations were reported for Black Sudanese (Sandholzer et al. 1991) and Gabonese Bantu (Schmidt et al. 2006). Differences exist also within populations from major ethnic groups. In Europe, Finns have the lowest reported concentrations (Erhart et al. 2018; Waldeyer et al. 2017). Asian populations are even more heterogeneous. Most studies report very low levels and highly skewed Lp(a) distributions in East Asians (Japanese and Chinese). South-East Asians, e.g., Indians and Thais, have concentrations between Europeans and East Asians and Africans (Schmidt et al. 2016; Sandholzer et al. 1991; Helmhold et al. 1991; Enkhmaa et al. 2016) (Fig. 3.1). Notably different Lp(a) concentrations have also been reported for the same or similar population, e.g., Chinese (Sandholzer et al. 1991; Helmhold et al. 1991; Enkhmaa et al. 2016).

G. Utermann (✉)
Institute for Genetic Epidemiology, Medical University of Innsbruck, Innsbruck, Austria
e-mail: gerd.utermann@i-med.ac.at

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_3

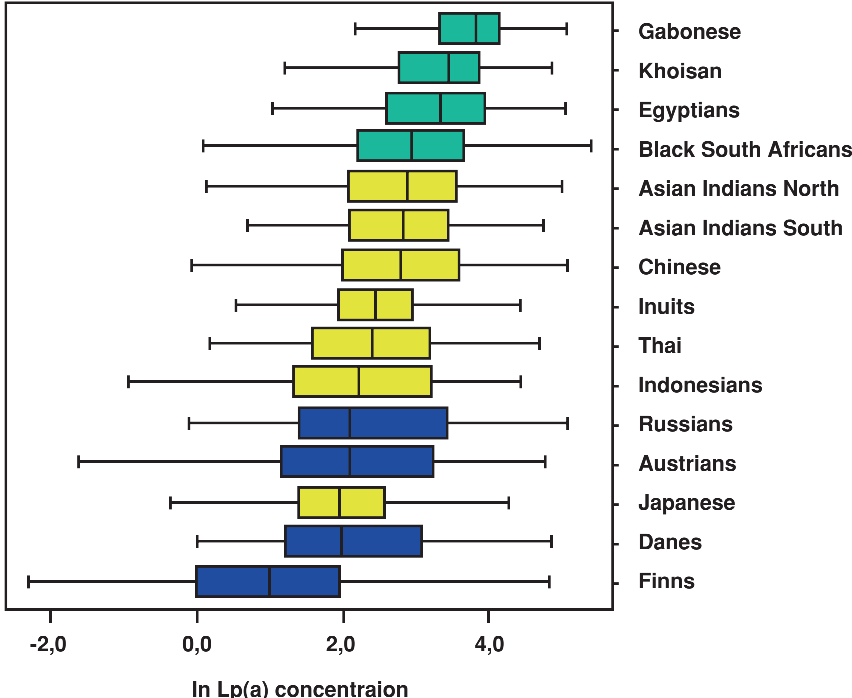


Fig. 3.1 Distribution of Lp(a) concentrations in 15 populations. Median, range, and 95% confidence intervals are given (in ln mg/dL). Colors denote continental groups. (Data from Sandholzer et al. 1991; Schmidt et al. 2006; Kraft et al. 1996; Khalifa et al. 2015; Scholz et al. 1999; Trommsdorff et al. 1995)

It is unclear whether this reflects heterogeneity in large populations (e.g., Chinese from Singapore vs Hongkong) or differences in assay methods.

In healthy individuals, Lp(a) concentrations remain rather constant over time though they may fluctuate moderately. One reason is the effect of hormones which result in changes during puberty and pregnancy (Kostner and Kostner 2004) but environmental factors like nutrition have little effects and can be neglected here. Twin studies resulted in heritability estimates of $h^2 > 90\%$ in Europeans (Austin et al. 1992; Boomsma et al. 1993). How could this trait be viewed as a simple dominant and how is the quantitative trait genetically controlled? Both questions can be answered today. The answer to the first question is simple. The presence of Lp(a) in human serum or plasma was initially shown by an immunological test. The low sensitivity of the test resulted in a Lp(a)-positive reaction only in individuals with higher Lp(a) concentrations in plasma. The high Lp(a) concentrations were inherited in families in a dominant fashion. Depending on the sensitivity of the antiserum, more or less individuals were tested "positive" which explains why different frequencies of Lp(a)+ were reported by researchers at that time (Wendt 1967). The answer to the second question will be the major topic of this review.

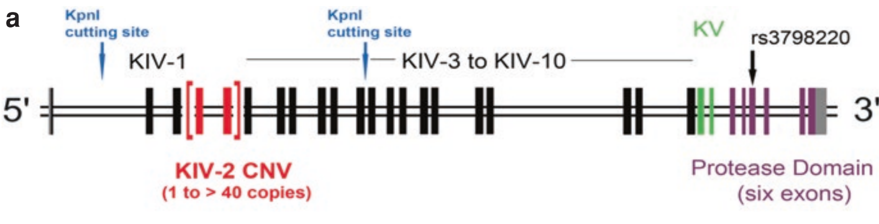
Structure of Lp(a) and *LPA* Gene

For understanding the genetics of the Lp(a) trait, a brief description of the structure of Lp(a) is necessary. Lp(a) is a complex, assembled from one low-density lipoprotein (LDL) and the high molecular weight glycoprotein apolipoprotein(a) [apo(a)] which confers the immunological specificity to the particle. Both are held together by non-covalent binding of domains in apo(a) to apoB in LDL and by covalent binding through a single disulfide bridge between apo(a) and apoB (Schmidt et al. 2016; Brunner et al. 1993; Koschinsky et al. 1993; McCormick et al. 1995; Callow and Rubin 1995; Ernst et al. 1995; Gabel and Koschinsky 1998). Apo(a) is highly glycosylated, does not bind lipids, and is not a true apolipoprotein. The protein has a high homology to plasminogen from which it has evolved during primate evolution by a series of gene duplication, deletions, domain duplications, and point mutations (McLean et al. 1987; Tomlinson et al. 1989). The result is an odd protein consisting of a signal sequence, ten different domains with homology to *PLG* kringle type IV (KIV-1 to KIV-10), one *PLG*-derived kringle type V(KV), and a protease domain with AA-substitutions rendering it inactive toward plasmin substrates.

LPA KIV-2 VNTR and Lp(a) Concentration

One of the kringles, KIV-2 occurs in multiple identical copies and varying copy numbers in the gene (Utermann 1989; Lackner et al. 1991, 1993; Boerwinkle et al. 1992; Kraft et al. 1992) (Fig. 3.2). The genomic size of one KIV-2 unit is 5.6 kb. This variable number of repeats (VNTR) is translated and transcribed into protein resulting in a size polymorphism of apo(a) (Schmidt et al. 2016; Utermann 1989; Utermann et al. 1987). The size polymorphism of LPA/apo(a) has been demonstrated at the protein level by polyacrylamide- or agarose-gel-electrophoresis/Western blotting of plasma using antibodies against apo(a)/Lp(a) (Fig. 3.3a) or at the DNA level by enzymatic digestion of genomic DNA using appropriate DNAses followed by pulsed-field-gel electrophoresis and Southern blotting with KIV-2-specific probes (Fig. 3.3b). Using enzymes, e.g., KpnI that cut the genomic DNA only outside the KIV-2 repeats (Fig. 3.3), the complete block of DNA can be cut out and the number of repeats determined from its size (Lackner et al. 1991; Boerwinkle et al. 1992; Kraft et al. 1992). A technique, which has been used only in one single publication and needs special skills in molecular cytogenetics, is fiber-FISH. This enabled to visualize and count the number of KIV-2 repeats of single alleles under the microscope (Erdel et al. 1999) (Fig. 3.3). The frequency of KIV-2 alleles varies significantly between different ethnic groups (Fig. 3.4a).

The KIV-2 VNTR held the key to the understanding of the genetics of the Lp(a)-trait. The size of apo(a) isoforms was shown to be inversely correlated with Lp(a) concentration in plasma (Fig. 3.4b). On average, small isoforms corresponding to low KIV-2 copy number were associated with high Lp(a) in plasma and large



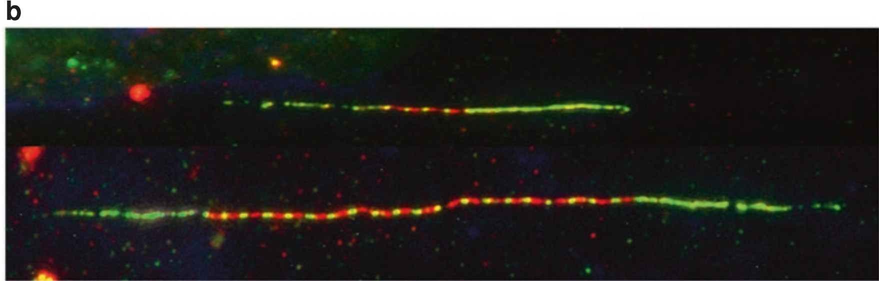


Fig. 3.2 Panel (**a**): Exon-Intron structure of the human *LPA* gene. Domains are represented in different colors (KIV-2 = red; KIV-1 and KIV-3 to KIV-10 = black; KV = green; protease domain purple). Indicated are the KIV-2 VNTR, cutting sites for KpnI (Lackner et al. 1991) and rs3798200 (Clarke et al. 2009) (Adapted from Noureen et al. 2015 with permission). Panel (**b**): Fibre-FISH image of *LPA* alleles with four and nineteen KIV-2 repeats (colored in red-yellow; count yellow dots flanked by red). (Copy of Fig. 1b in Erdel M et al. Nat. Genet 1999; **21**:357–358)

isoforms (high KIV-2 copy numbers) with low concentrations (Schmidt et al. 2016; Utermann et al. 1987). There is, however, wide variation within alleles defined by copy number (allele-associated Lp(a) levels) especially for low copy number alleles (Fig. 3.5).

The analytical techniques agarose-gel-electrophoresis/Western blotting and PFGE/Southern blotting achieve a similar resolution and with both >30 alleles of different sizes have been demonstrated (Schmidt et al. 2016). They are, however, not equivalent but rather complement each other. By PFGE/Southern blotting the KIV-2 genotype can be precisely determined and >95% of individuals were found to be heterozygotes. It can, however, not be measured which concentration of Lp(a) is associated with each allele. By contrast, agarose-gel-electrophoresis/Western blotting allows assignment of Lp(a) concentration to both alleles if total Lp(a) concentration is known. Due to the extremely wide range of concentrations and the sensitivity limits of Western blotting, apo(a) alleles associated with very low or absent Lp(a) in plasma cannot be seen (so-called "null" alleles). Hence, in a considerable number of samples, only one isoform is seen on the blot. Further for such samples, it cannot be distinguished whether they are from a rare homozygote or from individuals with one "null" allele. The frequency of "null" alleles has been estimated from 1% to 29% depending on the population and sensitivity of Western blotting (Kraft et al. 1996; Marcovina et al. 1996; Gaw et al. 1994) which is interesting in view of the frequency of true "null" alleles defined today at the molecular level (Ogorelkova et al. 1999; Di Maio et al. 2020; Morgan et al. 2020; Mukamel et al. 2021).

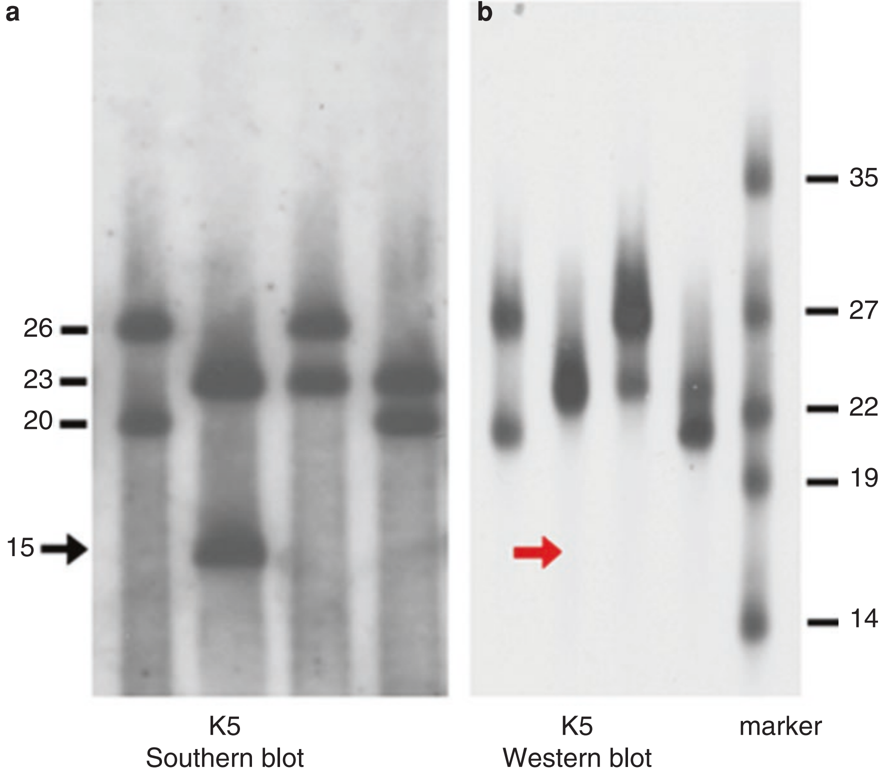


Fig. 3.3 Determination of the Size of Alleles of the KIV-2 VNTR by PFGE/Southern Blotting (Panel **a**) and Western Blotting (Panel **b**). The same four samples from one family were analyzed. The allele with 15 KIV repeats (corresponding to 6 KIV-2 repeats) in the lane denoted K5 in the Southern blot by a black arrow is not expressed (=null allele). The corresponding isoform is missing in the Western blot (red arrow). (Modified from Noureen et al. 2015 with permission)

Only the simultaneous application of both analytical techniques allows for a complete picture of the KIV-2 size polymorphism and its association with Lp(a) concentrations in plasma and the identification of "null alleles" (Fig. 3.3). Few such studies have been performed to date because they require carefully prepared intact DNA and the application of the laborious technique of PFGE/Southern blotting for large samples (Kraft et al. 1996; Gaw et al. 1994). These studies demonstrated significant differences in Lp(a) levels and KIV-2 allele and isoform frequencies and the relation of KIV-2 alleles with Lp(a) concentrations between major human ethnic groups. Importantly differences in KIV-2 allele frequencies did not explain the large differences in Lp(a) levels especially the much higher Lp(a) levels in Africans which was consistently observed in all studies (Schmidt et al. 2016; Kraft et al. 1996; Gaw et al. 1994). To circumvent the obstacles of the laborious DNA typing by PFGE-Southern blotting, researchers have used proxy values in epidemiological studies. In some, the total number of KIV-2 repeats was determined by quantitative

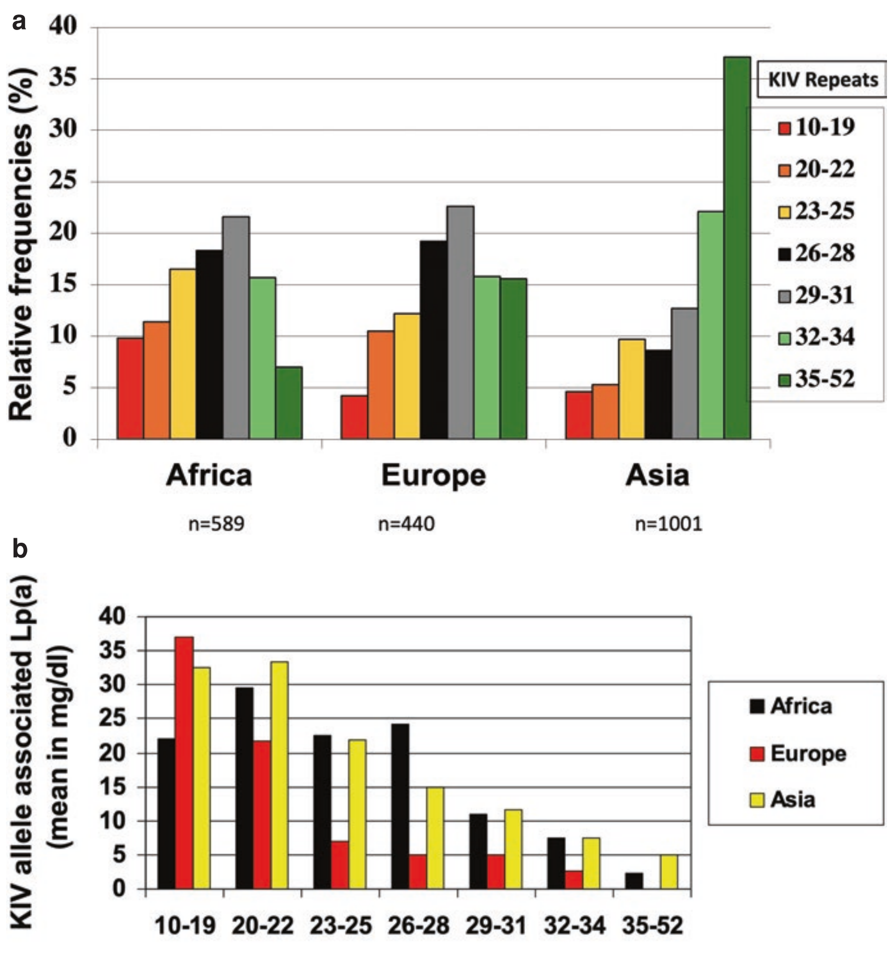


Fig. 3.4 Panel (**a**): Frequency distribution of binned KIV-2 VNTR alleles (numbers denote KIV repeats including KIV-1 and KIV-3 to 10) in pooled data from three continental groups (Adapted from Schmidt et al. 2016 with permission). Panel (**b**): Lp(a) concentrations by ancestry associated with binned KIV-2 VNTR alleles. Note large differences in concentrations of Lp(a) associated with KIV-2 alleles of the same binned size category between the major continental groups. (Data from Schmidt et al. 2006; Kraft et al. 1996; Scholz et al. 1999)

PCR (qPCR) (Kamstrup et al. 2009). This allowed neither identification of the genotype nor assignment of allele-associated Lp(a) concentrations. Second, SNPs in the *LPA* gene (see below) in LD with the KIV-2 repeats were used as proxies (Clarke et al. 2009). Both approaches allow to detect strong associations. A serious caveat is, however, that LDs of SNPs with KIV-2 alleles may differ significantly between ethnic groups and use as proxy for Lp(a) concentration may lead to grossly false

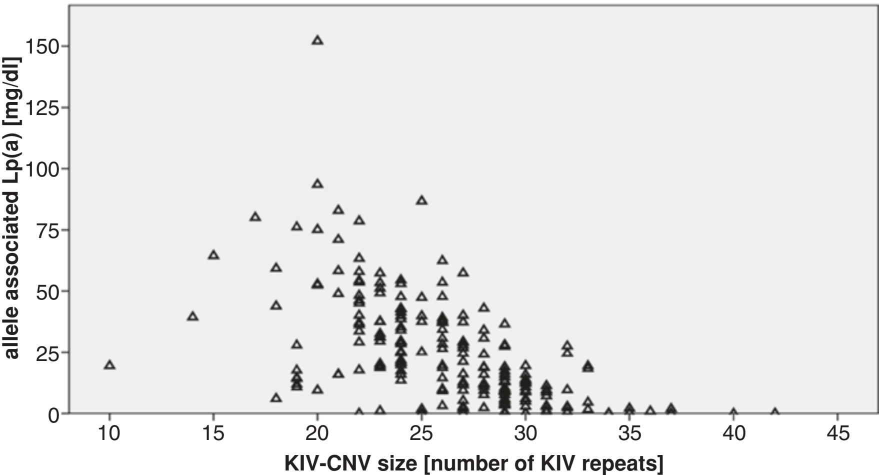


Fig. 3.5 Illustration of the inverse correlation of KIV-2 VNTR allele size with Lp(a) concentration (allele-associated Lp(a) concentration) in Gabonese Bantu. (Data from Schmidt et al. 2006. Adapted from Schmidt et al. 2016 with permission)

results. This has been demonstrated for rs3798220 which is associated with low KIV-2 copy number and high Lp(a) in Europeans (Clarke et al. 2009) and median/high copy numbers and low Lp(a) in East and South East Asians (Khalifa et al. 2015) (Fig. 3.6).

The apo(a) VNTR explains about 40–70% of the heritability of the quantitative Lp(a) trait depending on study design and population (Schmidt et al. 2006; Boerwinkle et al. 1992; Kraft et al. 1992). The KIV-2 VNTR was further used in family and sib-pair linkage studies to estimate the heritability of Lp(a) explained by the *LPA* locus. This demonstrated that heritability ranged from about 70% to >95% in populations of European descent (Boerwinkle et al. 1992; Kraft et al. 1992). *LPA* is also the major locus determining Lp(a) levels in Africans but explained heritability is lower in Africans than in populations of European descent (Schmidt et al. 2006; Mooser et al. 1997; Scholz et al. 1999; Enkhmaa et al. 2019). Hence, it appears that both the KIV-2 VNTR and the *LPA* locus explain less of the genetic variability of Lp(a) in Africans suggesting that other loci or environmental factors have a larger impact on Lp(a) levels in Africans. Several GWAS confirmed that *LPA* is the major locus determining Lp(a) levels in Europeans (Mack et al. 2017; Li et al. 2015; Lu et al. 2015; Ober et al. 2009). Minor loci detected by GWAS are the genes coding for apo E (Mack et al. 2017) and apo H (Hoekstra et al. 2021). ApoH codes for beta-2 glycoprotein 1 which has been shown to physically interact with apo(a) in human plasma (Köchl et al. 1997) and which has been implicated in the pathogenesis of anti-phospholipid syndrome. A candidate association study implicated *TLR2* as a gene modulating Lp(a) levels (Mack et al. 2017). The genomic heritability, i.e., the heritability explained by the measured genetic variation in a GWAS was estimated to account for 49.5% of the total variability of Lp(a) levels (Mack et al. 2017).

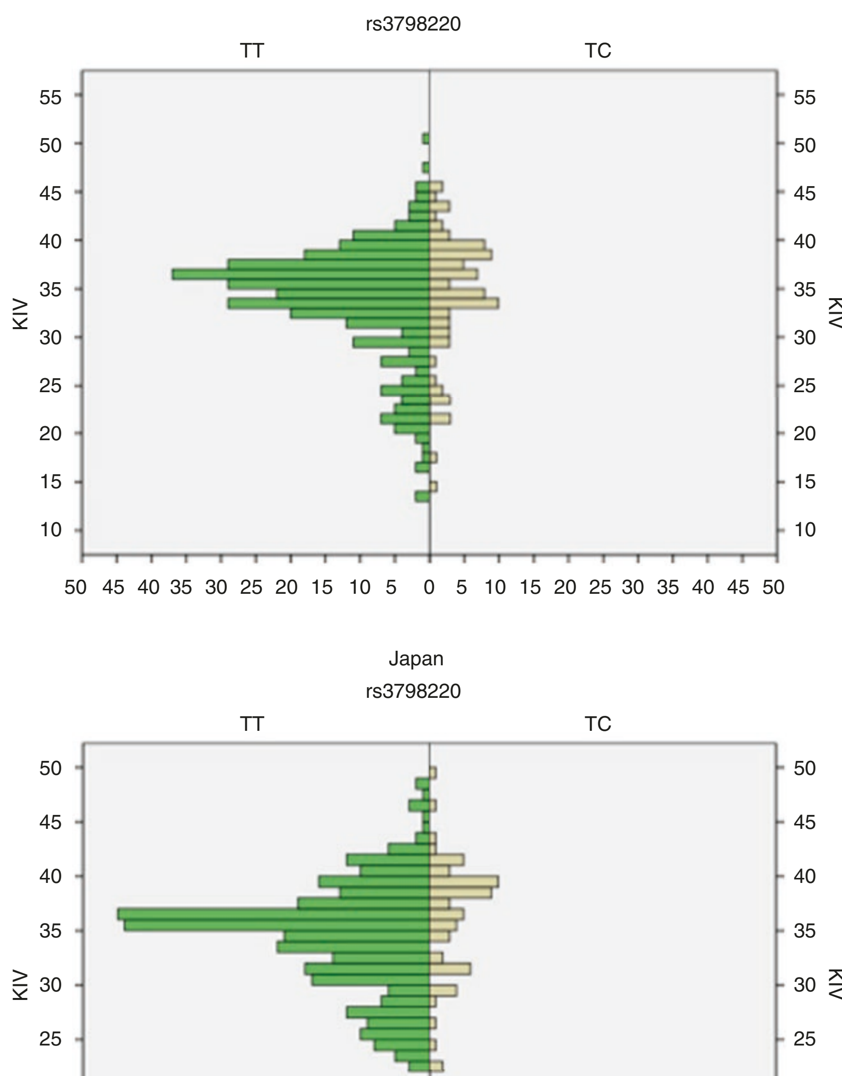


Fig. 3.6 Graphic representation of the distribution of SNP rs3798220 which is associated with short KIV-2 alleles in Europeans (Clarke et al. 2009) in Chinese and Japanese were it is associated with long KIV-2 alleles. (Calculated from the data of Khalifa et al. 2015)

Causal Effect of the KIV-2 VNTR on Lp(a) Concentration

Genetic studies, e.g., family/sib–pair linkage and analysis by a variance components model can provide estimates on the magnitude of the heritability explained by a variant or locus but cannot provide evidence that the variation itself is causal. For the KIV-2 VNTR causality was demonstrated in cell culture experiments. Transient and stable expression in the human hepatocarcinoma cell line HepG2 of recombinant apo(a) which differed only in the number of identical KIV-2 domains showed that the amount of apo(a) secreted into the culture media correlated inversely with the number of KIV-2 repeats in the recombinant isoform mimicking the situation in plasma (Brunner et al. 1996; Lobentanz et al. 1998). This was due to differences in processing of the translated proteins in the endoplasmic reticulum (ER). For large isoforms, a predominant apo(a) precursor protein was retained in the ER and little mature protein secreted from the cells. For small isoforms, most protein was secreted into the cell media. The mature form was present only in low levels in the Golgi apparatus. Temperature blocking experiments showed that no apo(a)/apoB complexes can be demonstrated inside the cells (Lobentanz et al. 1998). Studies in primary baboon hepatocytes came to the same conclusion (White et al. 1993, 1994). Steady-state labeling and pulse chase experiments demonstrated that the residence time of an isoform in the ER is determined by its size. Together the experiments in human and baboon cell cultures have shown that the efficiency of post-translational processing of apo(a) is a major determinant of Lp(a) plasma levels and that the contribution of the KIV-2 repeat is causal. Considering that the structure of each kringle in mature apo(a) is stabilized by three internal disulfide bonds and one N-linked glycosylation site, it is reasonable to assume that the more kringles are present in an isoform the more difficult it becomes for a cell to fold correctly. White et al. (1994) also studied processing and secretion of so-called transcript positive null alleles in the primary baboon hepatocytes and observed retention and degradation of the protein in the cell. Together with in-vivo turnover experiments in humans which had demonstrated that Lp(a) plasma concentrations are determined by apo(a) synthesis (Krempler et al. 1980; Rader et al. 1993) and that apo(a) isoforms of different size are synthesized at different rates this clearly establishes apo(a) synthesis as the critical process determining the quantitative Lp(a) polymorphism.

Simple Repeats, RSPs, and SNPs in *LPA* and Their Effects on Lp(a) Levels

The discrepancy between the heritability explained by the VNTR and by the locus which exists in all studied populations needed an explanation and suggested further genetic variation at the *LPA* locus or nearby beyond the KIV-2 VNTR with effects on Lp(a) levels. Several polymorphisms including SNPs, simple repeats, or restriction site polymorphisms in *LPA* were shown to explain some of the “missing

heritability" of Lp(a) levels. A pentanucleotide repeat (PNRP) at the 5′ at −1374 of the *LPA* locus explained 15% of the variability of Lp(a) levels in Europeans independent from the KIV-2 VNTR but none in Africans (Trommsdorff et al. 1995).

Expression of 105 kb 5′-flanking fragments containing the *LPA* promoter and the PNRP from 10 different alleles with 8 or 9 PNRs in HepG2 cells found equal promoter activity for all tested allelic fragments regardless whether they were from alleles associated with high or low Lp(a) in plasma (Bopp et al. 1995). A G>A polymorphism at −914 is also located in the tested fragments and had no effect on promoter activity. Variation in linkage disequilibrium with the PNRP and the −914G/A polymorphism has therefore to be implicated as responsible for the effects on Lp(a) levels.

Several non-synonymous SNPs were detected in the "unique" kringles KIV-3 to 10 by sequencing of exons and flanking regions (Ogorelkova et al. 2001; Prins et al. 1997, 1999; Crawford et al. 2008). Some had dose-dependent effects and some, e.g., KIV-8 T23P (also called T12P) were predicted by bioinformatic tools like PolyPhen to have effects on Lp(a) levels (Crawford et al. 2008). They were detected in populations from Africa, Europe, and North Americans of African, Mexican, and European descent. Most were present in only one ethnic group and none in all (Ogorelkova et al. 2001; Dumitrescu et al. 2011).

This is clear evidence that the genetic architecture of the Lp(a) trait differs substantially between human ethnic groups, a conclusion consistent with recent genomic analysis (Mukamel et al. 2021).

Only for few SNPs functional data were provided. One is the donor splice site variant KIV-8+1G>A (rs41272114) which has a carrier frequency of 6% in Europeans (Ogorelkova et al. 1999). The variant codes for a truncated form of apo(a) which lacks the site for formation of the covalent disulfide bridge with apoB in LDL and prevents assembly of the Lp(a) complex. The free truncated form of apo(a) is fragmented in plasma resulting in Lp(a) deficiency in homozygous carriers (Ogorelkova et al. 1999). The variant was observed in similar frequency in Austrians and Finns (Ogorelkova et al. 1999; Lim et al. 2014) and was used in Mendelian randomization approaches to demonstrate that genetically reduced Lp(a) levels result in a reduced risk for CHD (Lim et al. 2014; Kyriakou et al. 2014).

A nonsense mutation (R21X) was detected by cloning and sequencing of KIV-2 (Parson et al. 2004) from members of a family segregating a "null" allele and its carrier frequency determined by a sophisticated PCR protocol. Western blotting of plasma from family members carrying the variant demonstrated a truncated isoform. The variant had a low carrier frequency of 2% in Austrians. Recently, Di Maio et al. (2020) investigated the R21X variant in >10.000 individuals from three European population samples and determined carrier frequencies from 1.6% to 2.1%. The variant was associated with KIV-2 alleles of medium copy number and mean Lp(a) levels in carriers were −11.7 mg/dL lower than in non-carriers. The frequency distribution differed between world populations. According to data from

the 1000 Genomes project, the variant is present in Europeans and South-East Asians, occurs with varying frequency in South Americans, and is absent in Africans (Di Maio et al. 2020).

Surprisingly, the R21X variant was found to be present on the same allele as the null mutation KIV-8+1G>A (rs41272114). All alleles carrying the R21X mutation also carried the null mutation KIV-8+1G>A but not vice versa suggesting that KIV-8+1G>A is the older mutation and R21X occurred on an allele with the splicing defect in KIV-8 generating a "double null" variant (Di Maio et al. 2020).

Only three non-synonymous variants have been demonstrated by functional studies to have effects on plasma Lp(a) levels. The variant I4399M (rs3798220) in the protease domain of *LPA* has been associated with elevated Lp(a) and CHD risk (Shiffman et al. 2008; Luke et al. 2007) and a benefit from aspirin therapy was reported (Chasman et al. 2009). An effect of the variant on fibrin clot architecture and fibrinolysis has been suggested (Shiffman et al. 2008; Luke et al. 2007) but this was not confirmed in all populations and a dependency from ethnicity was postulated (Rowland et al. 2014). McCormick and coworkers (Morgan et al. 2020) carefully characterized two non-synonymous SNPs R990Q and R1771C, which both result in a null phenotype. They occur in positions of *LPA* which are homologue to positions in *PLG* where mutations result in PLG deficiency. The positions are important for proper folding of the protein and variants poorly transit to the Golgi and are not secreted (Morgan et al. 2020).

With the exception of the R21X variant, which had been detected by analysis of a single family, the KIV-2 VNTR had remained a black box for mutation detection and was not accessible by standard sequencing nor next-generation sequencing (NGS). Depending on the number of identical KIV-2 repeats, this region can include up to 70% of the coding sequence of the *LPA* gene. This region therefore may significantly contribute to functionally relevant variation in the gene. A single study using a laborious cloning- and a protocol for specific batch-wise PCR-amplification of KIV-2 repeats from alleles separated by PFGE detected several previously unreported variants in the KIV-2 repeats including a donor splice site mutation designated K421+1G>A which was associated with a "null allele" (Noureen et al. 2015) (Fig. 3.3a). This variant occurred in two African and one European alleles. A putative acceptor splice site variant K422-6T>G associated with short alleles was present with high frequency (10% in Khoi San to 40% in Egyptians) only in African samples. Due to the small total number of only 90 alleles from six ethnic groups, the study was limited and exact population frequencies and data on effects of variants on Lp(a) levels in populations were not provided.

Coassin et al. (2017) used the batch amplification of KIV-2 in combination with NGS. Starting with a discovery set of samples from individuals with discordance between KIV-2 copy number and Lp(a) levels, they identified a novel frequent splice site variant G4925A. The variant results in a reduction in splicing activity in

an in vitro assay but not a “null allele.” 4925G>A has a carrier frequency of 21% in Europeans is associated with short repeats (mainly 19–25 K-IV repeats). It reduced Lp(a) levels by 31.8 mg/dL and coronary risk significantly (Coassin et al. 2017).

Only recently, a pipeline for ultradeep sequencing of the KIV-2 repeat domain of *LPA* (Coassin et al. 2019) and methods to measure KIV2 VNTR length from whole-exome sequencing data has been developed and allowed for the systematic investigation of variation in this genomic region. The effect of a splice site variant 4733G>A detected in this study on Lp(a) levels and CVD was studied in detail (Schachtl-Riess et al. 2021) together with the previously reported splice site variant 4925G>A (Coassin et al. 2017). The 4733G>A allele had a high carrier frequency of 38% and occurred on KIV-2 repeats of all sizes. Overall, it reduced Lp(a) levels by 13.6 mg/mL. The two splice site variants cooperate in their effect on Lp(a) levels and CHD risk reduction (Schachtl-Riess et al. 2021).

A further possible level of complexity of the genetic architecture of the Lp(a) trait is the presence of cis-epistatic effects of variants on Lp(a) levels. A GWAS of DNA methylation identified a novel association signal associated with elevated Lp(a) levels in the *LPA* promoter (Coassin et al. 2020). The effect turned out to be caused by a non-methylated SNP (rs10455872) which is in LD with short KIV-2 alleles (Coassin et al. 2020). A cis-epistatic effect on Lp(a) levels and coronary risk was recently demonstrated for variants rs1800769 and rs9458001 which are jointly associated with elevated Lp(a) levels and with risk for CHD (OR 1.37). Most of this effect was however explained by rs140570886 (Zeng et al. 2022) known to be associated with Lp(a) levels (Mack et al. 2017).

An epistatic effect of two SNPs and the KIV-2 VNTR was also noted in the study by Mukamel et al. (2021). These authors estimated KIV-2 VNTR copy number from whole genome sequencing data. Fusing these data by imputation with SNP data, they were able to define KIV-2 haplotypes and estimate their effects on Lp(a) concentrations. They identified 17 protein altering variants. Six of the variants abolished splice sites totally or partially and six were missense variants, all of which greatly reduced Lp(a) levels. Most of these variants were detected in the KIV-2 VNTR. Variants resulting in increased Lp(a) concentration were found in the 5′UTR of the *LPA* gene (Mukamel et al. 2021).

The work of Mukamel et al. (2021) also provided new insights into the genetic basis underlying the differences in Lp(a) levels between human ethnic groups in particular between sub-Saharan Africans and Europeans. Analysis by ancestry demonstrated that these differences are largely explained by a significantly lower frequency of Lp(a) decreasing variants and higher frequency of Lp(a) increasing SNPs in Africans compared to Europeans (Fig. 3.7).

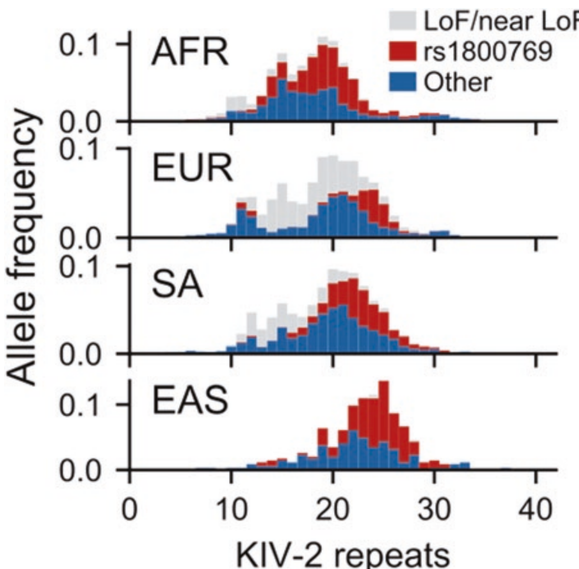


Fig. 3.7 Illustration showing the association of loss-of-function (LoFs) SNPs and Lp(a) increasing SNP rs1800769 in the 5′UTR of the *LPA* gene with KIV-2 VNTR alleles by ancestry. Note the excess of LoFs and decrease of rs1800769 in Europeans. (From Mukamel et al. 2021 with permission)

Summary

The presently available knowledge of the genetic determination of Lp(a) levels in plasma is summarized in Fig. 3.8. The two *LPA* alleles in an individual determine Lp(a) concentration in a codominant manner. The concentration conferred by each allele (allele-specific concentration) depends on the number of KIV-2 repeats (KIV-2 VNTR allele) which determine secretion rates of apo(a) from liver cells and SNPs effecting Lp(a) concentration in the *LPA* allele. Most functionally characterized SNPs with causal effects described to date are loss of function or nonsense variants which reduce Lp(a) concentration in carriers. Most SNPs with strong effects on Lp(a) are restricted to one or few ancestries. Cis-acting epistatic effects between SNPs and between SNPs and alleles of the KIV-2 VNTR have also been described.

The high numbers of KIV-2 VNTR alleles and SNPs effecting Lp(a) concentration which occur in different allelic associations result in an allelic series with numerous alleles where each allele has an individual effect on Lp(a). The frequency distributions of the *LPA* alleles determine Lp(a) level distributions in populations. The up to fourfold differences in median Lp(a) concentrations and of the distributions of Lp(a) levels between ethnic groups are not explained by the KIV-2 VNTR

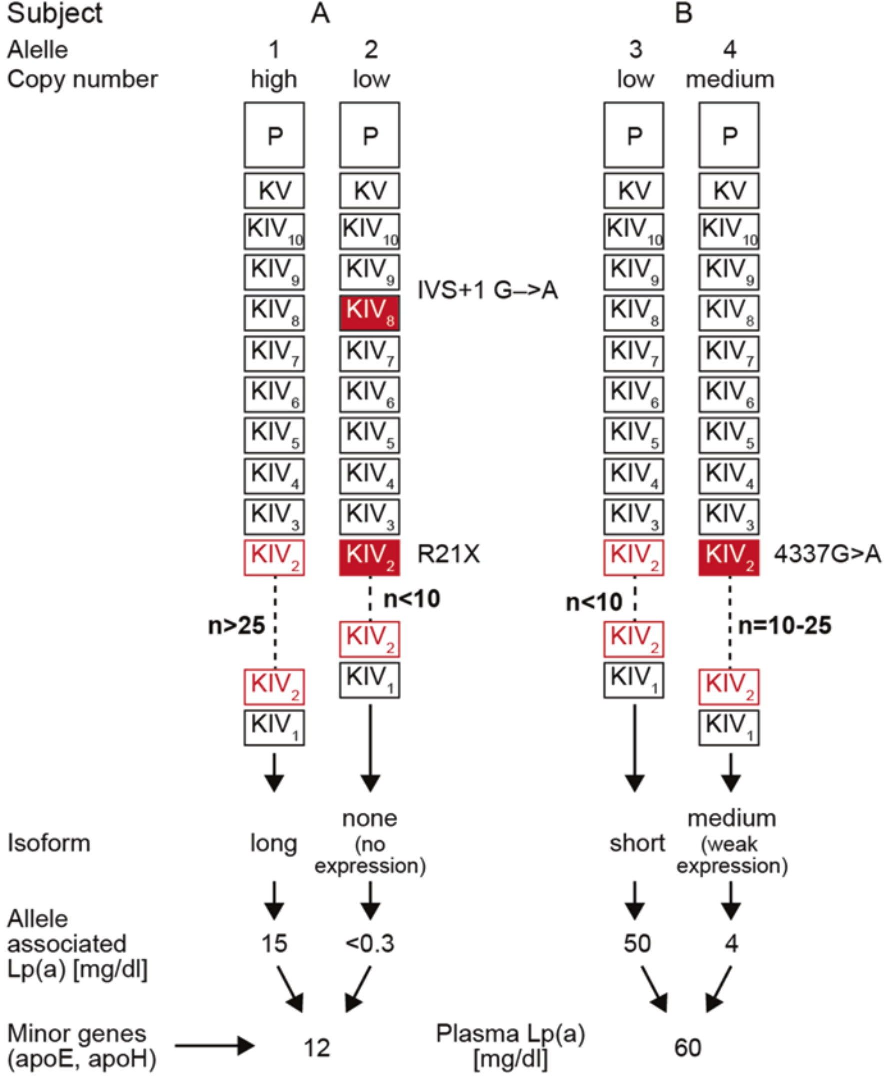


Fig. 3.8 Illustration of the genetic determination of Lp(a) concentrations in plasma by the combined effects of the KIV-2 VNTR and SNPs in the *LPA* gene. The number of KIV-2 repeats determines apo(a) isoform size and correlates inversely with the rate of synthesis and with Lp(a) concentration in plasma. Allele 1 in subject A codes for a long isoform and moderately low Lp(a) and allele 3 in subject B for a short isoform and high Lp(a). This basic situation is modulated by SNPs. As examples allele 2 in subject A carries the Lp(a) decreasing SNPs KIV-8 IVS+1G>A (Ogorelkova et al. 1999) and KIV-2 R21X (Parson et al. 2004) which are in strong LD (Di Maio et al. 2020) and result in a null allele and allele 4 in subject B which codes for an isoform of intermediate size and carries the variant 4733G>A in KIV-2 which affects splicing and moderately decreases Lp(a) (Schachtl-Riess et al. 2021). The total plasma Lp(a) concentration in a subject is the sum of the two allele-associated concentrations (cis-epistatic effects are not considered). Other loci may have minor effects by unknown mechanisms

but rather by different types, allele distributions, and LDs with KIV-2 alleles between them. Other gene loci beyond *LPA,* i.e., *APOE* and *APOH* have only small effects on Lp(a) concentrations.

Acknowledgments I thank Anita Neuner for help with the literature, Eugen Preuss for preparing figures, and Florian Kronenberg for his support and for critically reading the manuscript.

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Chapter 4
Lp(a) Metabolism

John S. Millar and Daniel J. Rader

Introduction

Lp(a) is a lipoprotein of unknown function that is an important causal factor in atherosclerotic cardiovascular disease (ASCVD) and aortic valvular stenosis. Plasma levels of Lp(a) are highly genetically determined, and the distribution of plasma levels of Lp(a) in the general population is skewed to the left with about one quarter of individuals having elevated levels that put them at increased risk of cardiovascular disease (Varvel et al. 2016).

Lp(a) consists of an LDL-like lipoprotein containing apolipoprotein (apo) B100 that is bound to apo(a), a highly glycosylated protein of variable length (Schmidt

J. S. Millar
Division of Translational Medicine and Human Genetics, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Institute for Diabetes Obesity and Metabolism, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA
e-mail: jsmillar@pennmedicine.upenn.edu

D. J. Rader (✉)
Division of Translational Medicine and Human Genetics, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Institute for Diabetes Obesity and Metabolism, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Department of Genetics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Institute for Translational Medicine and Therapeutics, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA

Smilow Center for Translational Research, University of Pennsylvania, Philadelphia, PA, USA
e-mail: rader@pennmedicine.upenn.edu

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_4

et al. 2016). The apo(a) peptide consists of a series of domains that are highly homologous to several domains of plasminogen (McLean et al. 1987). The N-terminal portion of apo(a) consists of a variable number of repeating domains that are homologous to kringle IV of plasminogen. This is followed by a non-variable portion that consists of two additional domains that are homologous to the kringle V and the serine protease domains of plasminogen. The number of kringle IV repeats in the variable portion of apo(a) range from 2 to more than 40 resulting in over 30 different isoforms ranging in size from approximately 300 to 800 kDa (Kronenberg and Utermann 2013).

While the vast majority of apo(a) in blood is found covalently bound to apoB100 on Lp(a) particles that overlap the LDL–HDL density range (Rainwater et al. 1995), it has been noted that a proportion of apo(a) can be found non-covalently associated with triglyceride-rich lipoproteins (Bersot et al. 1986). It is unclear if these complexes develop into mature Lp(a) particles. Such a model suggests that mature Lp(a) particles are formed extracellularly in plasma consistent with findings from some in vitro studies examining apo(a) secretion from hepatocytes (Koschinsky et al. 1991; White et al. 1993). However, there is a report showing evidence for apo(a) binding to apoB-containing lipoproteins intracellularly (Bonen et al. 1997) leading to an alternative model whereby Lp(a) can be formed intracellularly and secreted as an intact particle.

In addition to the apoB100 and apo(a) components, proteomic analysis of highly purified Lp(a) has identified 35 additional proteins that are associated with Lp(a) (von Zychlinski et al. 2011). In addition to proteins known to be involved in lipid metabolism (such as apoE and apoC-III), other proteins found associated with Lp(a) include those involved in wound healing (coagulation [fibrinogen], complement activation [complement C3 and C4A]), and inflammatory response (platelet activating factor acetyl hydrolase). However, biological significance of these additional proteins associated with Lp(a) is unknown.

While the Lp(a) resembles LDL containing a covalently linked apo(a) peptide, analysis of the lipid portion of Lp(a) has revealed that unlike LDL, Lp(a) is enriched in oxidized phospholipids (Tsimikas and Witztum 2008), both in the lipoprotein portion of the particle as well as being bound to the apo(a) peptide (Leibundgut et al. 2013). It has been shown that oxidized phospholipids are transferred from LDL to Lp(a) in vitro and proposed that Lp(a) is the preferential carrier of oxidized phospholipids in plasma (Bergmark et al. 2008). The presence of large amounts of oxidized lipids on Lp(a) could contribute to the atherogenicity of this lipoprotein. A genome-wide association study designed to identify risk factors for aortic stenosis identified variants at the *LPA* locus encoding apo(a) as the most significantly associated genomic locus. This has led to the hypothesis that oxidized phospholipids on Lp(a) contribute to the progression of aortic calcification and stenosis (Yeang et al. 2016).

The apo(a) peptide is encoded by the *LPA* gene located on chromosome 6q27 (Scanu et al. 1991). The gene is primarily expressed in liver with minor expression in kidney. The length of each *LPA* allele is variable due to there being variability in the copy number of domains that encode kringle IV type 2 (KIV-2) (Lanktree et al. 2010). The number of KIV-2 domain repeats in *LPA* has been estimated to range

from 2 to >40 copies (Kronenberg and Utermann 2013). Null alleles of *LPA* that encode a truncated apo(a) protein that is unable to bind covalently to apoB have been reported (Ogorelkova et al. 1999). In addition, *LPA* alleles with a large number of the KIV-2 domain repeats are unable to be secreted, presumably due to being unstable intracellularly, and are therefore also considered null alleles (White et al. 1994). In vitro studies suggest that the number of KIV-2 domain repeats are inversely associated with circulating Lp(a) levels due to the more efficient intracellular processing and secretion of smaller apo(a) isoforms (White et al. 1994).

A number of genome-wide association studies have been conducted that provide insight into the genes that regulate Lp(a) levels. Quantitively, genetic variation at the *LPA* locus itself is, by far, the most important factor influencing Lp(a) levels. Clarke et al. identified two SNPs (rs10455872 and rs3798220) at the *LPA* locus, which were associated with reduced KIV-2 copy number, small Lp(a) size, and increased Lp(a) levels (Clarke et al. 2009). Mack et al. identified 30 single nucleotide polymorphisms (SNPs) in the *LPA* gene, which either increased or decreased Lp(a) levels (Mack et al. 2017). They confirmed the two SNPs identified by Clarke et al. as well as other SNPs that were associated with the number of KIV-2 domain repeats that are inversely associated with Lp(a) levels. There was also an association with Lp(a) levels and the apolipoprotein E (*APOE*) gene, specifically with the *APOE2* allele being associated with decreased Lp(a) levels. Li et al. also found an association between a SNP in the *APOE* gene with Lp(a) levels (Li et al. 2015). Since apoE is an exchangeable apolipoprotein that binds to multiple lipoprotein receptors, it is possible that apoE on Lp(a) contributes to the clearance of Lp(a) from the circulation. There is also an association between the Toll-like receptor 2 (*TLR2*) gene and Lp(a) levels (Mack et al. 2017) leading to speculation that TLR2 may participate in Lp(a) clearance from plasma. TLR2 is known to bind lipopolysaccharide but, thus far, there have been no reports regarding the interaction between Lp(a) and TLR2.

Lp(a) Metabolism

The metabolism of Lp(a) is complex and has been the subject of intensive investigation. Here we review the major aspects of what has been reported regarding Lp(a) metabolism.

Studies Addressing Potential for VLDL, LDL, and Lp(a) Interconversion

The first study to examine the metabolism of autologous Lp(a) in humans was conducted by Krempler et al. (1978) to characterize the clearance and metabolic fate of Lp(a) in plasma. Lp(a) was isolated using a combination of ultracentrifugation, and

size exclusion chromatography was reductively methylated using [^{14}C]-formaldehyde and injected Lp(a) into four male subjects with moderate dyslipidemia. They determined an average fractional clearance rate for Lp(a) to be 0.378 pools/day that corresponds to a residence time of 2.6 days which they found was slightly shorter than the residence time of LDL reported in the literature. They also found that between 3% and 8% of labeled Lp(a) appeared in the LDL density range suggesting conversion of a small amount of Lp(a) to LDL, although they did not have a sufficient radioactive signal in the LDL fraction to state this with certainty.

Krempler et al. (1980) followed up their original studies by using a radioactive iodine tracer which gives a much higher specific activity of the Lp(a) tracer permitting a longer trace period as well as higher degree of sensitivity. This allowed them to address the question of whether there was a conversion of Lp(a) to LDL. They isolated Lp(a) from the study participants, radioiodinated it, and injected it either in an autologous or homologous fashion into nine subjects with a wide-range of Lp(a) levels. They then followed the clearance of the radiolabeled Lp(a) from plasma for up to 21 days. It was found that the radioactivity from the labeled Lp(a) injected stayed with the Lp(a) particle indicating that there is no interconversion of the apoB100 moiety between Lp(a) and LDL. This group also examined the potential of VLDL and LDL apoB100 to be the precursor of apoB100 in Lp(a) (Krempler et al. 1979). This was done by injecting radiolabeled VLDL and examining the appearance of radioactivity in LDL and Lp(a). They found that there was a precursor-product between VLDL and LDL with a large proportion of the radiolabeled VLDL appearing in LDL. However, there was essentially none of the radiolabeled VLDL appearing in the Lp(a) fraction. They concluded that VLDL and LDL are not precursors to Lp(a) and that Lp(a) is secreted as a separate lipoprotein.

Jenner et al. (2005) studied the metabolism of Lp(a) in mildly hyperlipidemic subjects with low, medium, and high levels of Lp(a) using endogenous labeling with a stable isotope labeled leucine tracer under continuously fed conditions. Lp(a) was isolated, and the leucine tracer enrichments in the apo(a) and apoB100 moieties of Lp(a) were measured. Kinetic data were analyzed using a multicompartmental model that allowed for independent production and clearance of apo(a) and apoB100 from Lp(a). They found the apoB100 portion of Lp(a) had a faster clearance than the apo(a) moiety suggesting that these components are metabolized differentially. A similar study by the same group, Diffenderfer et al. (2016), found that the clearance rate of apoB100 on Lp(a) was faster than that for the apo(a) component. It was also noted that the apoB100 moiety of Lp(a) had a tracer enrichment curve that differed from that of LDL apoB100, with tracer in apoB100 from Lp(a) appearing more rapidly than that from LDL B100. This would suggest that LDL apoB100 is not the direct precursor of apoB100 on Lp(a).

Demant et al. (2001) examined the kinetics of VLDL, IDL, LDL apoB100, and Lp(a) in relatively normolipidemic subjects using endogenous labeling with a leucine stable isotope tracer. The subjects were fasted for the first 10 h of the 12-day sample collection. The Lp(a) was isolated from plasma and then reduced so that the enrichment of the leucine tracer in the apo(a) and apoB100 moieties of Lp(a) could be measured. Kinetic data were analyzed using a multicompartmental model that

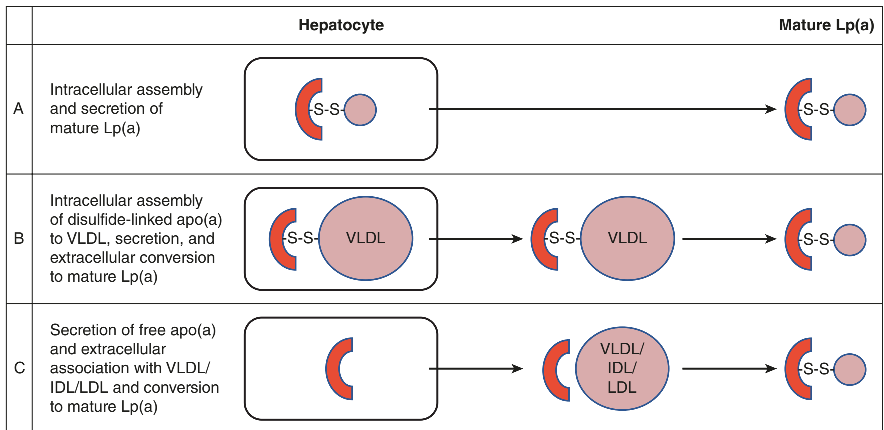

Fig. 4.1 The biosynthesis of mature Lp(a) may occur through diverse pathways. Possible pathways (not mutually exclusive) include: (**a**) intracellular assembly and secretion of the mature Lp(a) particle; (**b**) intracellular assembly of disulfide-linked apo(a) to VLDL, secretion, and extracellular conversion to mature Lp(a); (**c**) secretion of free apo(a) and extracellular association with VLDL/IDL/LDL and conversion to mature Lp(a)

allowed for formation of Lp(a) in both the liver (pre-formed) or in plasma. They calculated that about 50% of Lp(a) was formed in plasma from LDL with the remainder being secreted into plasma directly from liver as a preformed Lp(a) particle. They also compared the clearance rates of the apo(a) and apoB100 components of Lp(a) and found that they were cleared from plasma at similar rates. The potential pathways by which Lp(a) is formed from apo(a) and apoB-containing lipoproteins are shown in Fig. 4.1.

Studies Examining the Determinants of Lp(a) Concentration

Krempler et al. (1980) addressed the question of whether circulating Lp(a) levels were controlled primarily by production or by clearance. They measured Lp(a) production and clearance in subjects with a wide range of Lp(a) levels and found that there was a significant correlation between circulating Lp(a) levels and the Lp(a) production rate. There was no relationship between Lp(a) levels and the Lp(a) fractional clearance rate. These results indicated that Lp(a) levels are primarily controlled by production.

While circulating Lp(a) levels were known to be associated with apo(a) isoform size, it had been noted that there was a considerable variation in Lp(a) levels in subjects with the same apo(a) isoform size (Utermann et al. 1987). However, the mechanism responsible for these differences was unknown. Rader et al. (1993) examined the metabolism of Lp(a) in humans to determine the mechanism responsible for the differences in Lp(a) levels seen in individuals with the same sized

apo(a) isoform. Lp(a) was isolated from donors with a single apo(a) isoform size by sequential ultracentrifugation followed by density gradient ultracentrifugation. Isolated Lp(a) was then radiolabeled and injected into study subjects with a range of Lp(a) levels but having a single apo(a) isoform size. The results showed that there was no difference in the clearance of Lp(a) from plasma in subjects with the same apo(a) isoform size. However, there were substantial differences in the production rate of Lp(a) among individuals with the same sized apo(a) isoform, perhaps due to variants in the *LPA* gene that are independent of isoform size but which affect apo(a) production (White et al. 1994). They concluded that Lp(a) production is the most important determinant of the plasma level of Lp(a) independent of apo(a) isoform size.

It is well established that plasma Lp(a) levels are inversely correlated with apo(a) isoform size, but the mechanism behind this correlation was unknown. Rader et al. (1994) examined the physiology responsible for differences in Lp(a) levels based on apo(a) isoform size. The goal of the study was to determine if there were differences in the fractional clearance rates of Lp(a) particles containing different sized apo(a) isoforms. Healthy normolipidemic subjects were injected with either autologous or homologous radiolabeled Lp(a) isolated from plasma by sequential followed by density gradient ultracentrifugation. Subjects with different apo(a) isoform phenotypes were injected with radiolabeled Lp(a) preparations containing different sized apo(a) isoforms. They found that Lp(a) containing different sized apo(a) isoforms had similar clearance rates consistent with what they had observed in their previous study (Rader et al. 1993). However, there were substantial differences in the production rate of Lp(a) containing different sized apo(a) isoforms. The production rate of Lp(a) containing small apo(a) isoforms was considerably higher than that for Lp(a) containing large apo(a) isoforms. They concluded that the inverse association of plasma Lp(a) concentrations with apo(a) isoform size is not due to differences in the fractional clearance rates of Lp(a) containing different sized isoforms but rather the production rate. These studies provided in vivo evidence that supported earlier in vitro observations that smaller apo(a) isoforms are more readily secreted from hepatocytes, likely due to more efficient intracellular processing (White et al. 1994). The relationship between apo(a) isoform size and apo(a) production is shown in Fig. 4.2.

The Role of the LDL Receptor in Lp(a) Clearance

The receptor(s) responsible for Lp(a) clearance from plasma are currently unknown. Since Lp(a) contains apoB100, the ligand for the LDL receptor, the potential role of the LDL receptor in mediating Lp(a) clearance has been of great interest. One approach to address this question has been the use of genetics. In studies with patients with familial hypercholesterolemia (FH), Kraft et al. found a gene dosage effect of the LDL receptor gene (*LDLR*) on Lp(a) levels when controlling for *LPA* alleles (Kraft et al. 2000). A study conducted using the UK Biobank found that

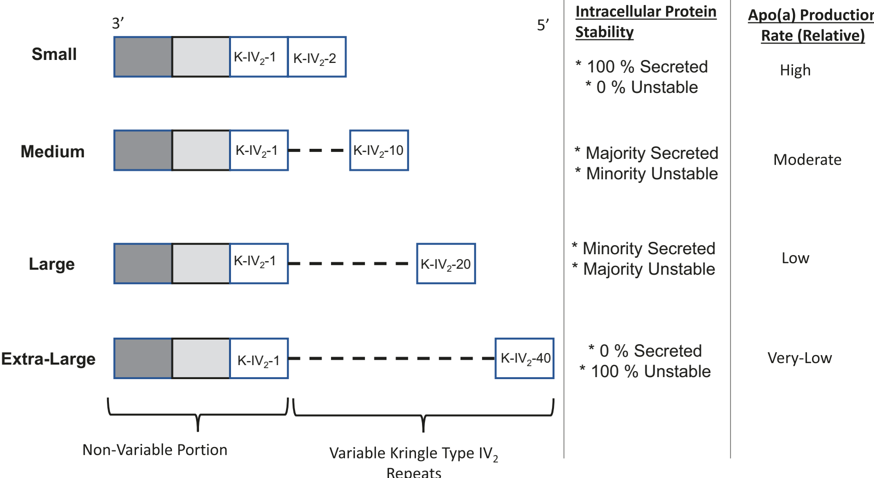

Fig. 4.2 The *LPA* gene encodes the apo(a) protein which is of highly variable length due to variation in the number of kringle IV2 domain repeats. The strong inverse association of apo(a) protein size with plasma Lp(a) level is due to the effect of apo(a) protein size on the rate of production, not catabolism, of Lp(a). While apo(a) peptide translation appears to occur normally, longer intracellular peptides are unstable and are targeted for pre-secretory degradation. While not shown here, in vivo evidence also indicates that variation in plasma Lp(a) levels among individuals with the same size isoform(s) is also due to differences in Lp(a) production, not catabolism

patients carrying variants in the LDL receptor that cause FH had higher Lp(a) levels than unaffected subjects (Trinder et al. 2020). However, it was determined that the population of FH patients studied was enriched in the rs10455872 SNP in *LPA* which is associated with higher Lp(a) levels; when the presence of the SNP was controlled for, it was found that Lp(a) levels were similar between patients with and without FH. Lp(a) levels are within the normal range in patients with familial defective apoB, a disorder where there is an amino acid substitution in LDL receptor binding domain of apoB (Innerarity et al. 1987). It is also of interest to note that genome-wide association studies have identified variants at the *LDLR* locus as being strongly associated with LDL cholesterol levels (Kathiresan et al. 2008); variants at the *LDLR* locus have not been found to be associated with Lp(a) levels (Clarke et al. 2009). Thus, the genetic data do not strongly support a role for the LDL receptor in directly mediating the catabolism of Lp(a).

There have also been experimental efforts to determine the role of the LDL receptor in Lp(a) clearance. Lp(a) can bind to the LDL receptor in vitro although the affinity of Lp(a) for the LDL receptor has been characterized as “weak” (Reblin et al. 1997). Knight et al. (1991) studied the role of the LDL receptor in the in vivo clearance of Lp(a) from plasma in hyperlipidemic patients with and without heterozygous FH. They radiolabeled autologous Lp(a) and LDL isolated by density gradient ultracentrifugation and examined the clearance of each from plasma. They found that there was no difference in the clearance rate of Lp(a) from plasma

between individuals with and without heterozygous FH despite there being significant differences in the clearance of autologous LDL. They also conducted in vitro studies that examined the ability of Lp(a) to compete with LDL binding to the LDL receptor. They found that Lp(a) was unable to compete with LDL for binding to the LDL receptor. They did find that after injection of radiolabeled Lp(a) that there was appearance of approximately 25% of the Lp(a) tracer in the LDL fraction which they interpreted as resulting from loss of apo(a) from the Lp(a) particle. They hypothesized that the resulting LDL could be cleared by LDL receptors. These authors also found no differences in the clearance of Lp(a) containing apo(a) isoforms of different sizes and that Lp(a) levels were correlated with the Lp(a) production rate.

Rader et al. studied the catabolism of Lp(a) in five patients with homozygous FH who had little to no LDL receptor function (Rader et al. 1995). Purified radioiodinated Lp(a) and LDL were simultaneously injected into homozygous FH patients and control subjects, and the catabolism was followed over time. While the catabolism of LDL was markedly delayed as expected, the catabolism of Lp(a) was not slower in homozygous FH patients than in control subjects. This study provided powerful evidence that the absence of a functional LDL receptor does not result in delayed catabolism of Lp(a) and suggested that the LDL receptor is not a physiologically important route of Lp(a) catabolism in humans.

In mice with marked overexpression of the LDL receptor, there was an increased uptake of Lp(a) levels resulting in decreased levels in plasma (Hofmann et al. 1990; Romagnuolo et al. 2017). Cain et al. addressed the question using mice deficient in the LDL receptor (Cain et al. 2005). When the catabolism of radiolabeled Lp(a) was studied in mice deficient in the LDL receptor compared to wild-type mice, there was no observed difference in Lp(a) turnover. This was consistent with the human studies and provided additional evidence that the LDL receptor is not a major contributor to Lp(a) clearance.

As another type of evidence, statins reduce LDL-C levels by causing the upregulation of the *LDLR* in hepatocytes and increased clearance of LDL and its precursors. However, as reviewed below, statins do not decrease Lp(a) levels and, if anything, cause Lp(a) levels to increase slightly. This also argues against the LDL receptor as being an important mediator of Lp(a) clearance. Overall, the data do not support an important role for the LDL receptor in mediating clearance of Lp(a) from blood.

The Role of Other Receptors in Lp(a) Clearance

The role of other receptors in Lp(a) clearance has been investigated and current information suggests that Lp(a) can be cleared from plasma through multiple receptors (Fig. 4.3). Available data suggest that apo(a) is the ligand responsible for receptor-mediated binding of Lp(a). For example, while excess LDL was shown to have minimal impact on Lp(a) clearance in mice, excess apo(a) significantly slowed

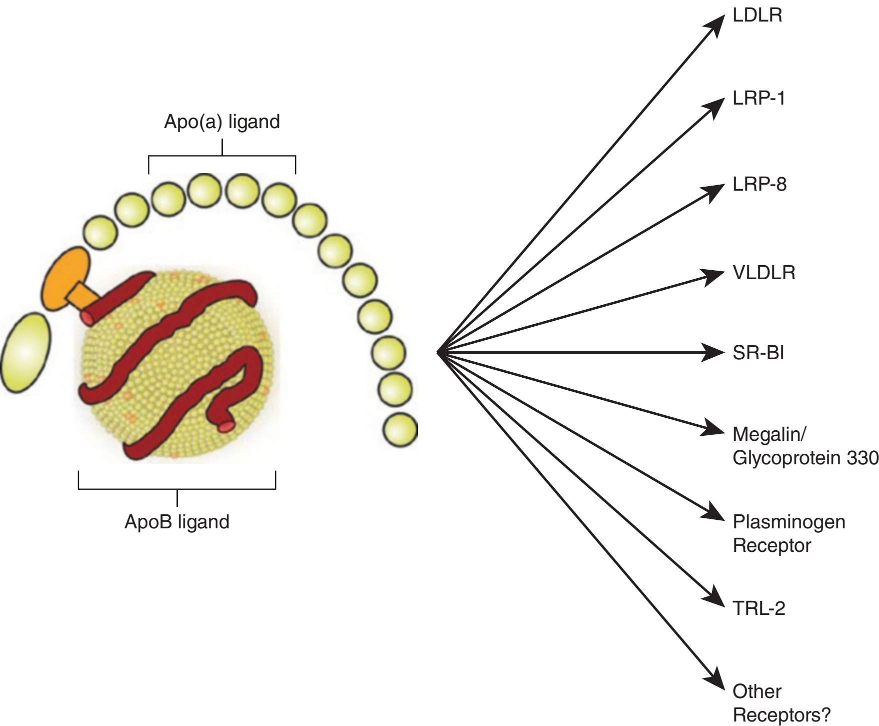

Fig. 4.3 Lp(a) clearance is not a major determinant of plasma Lp(a) levels and mostly occurs by the liver. The mechanisms of hepatic Lp(a) clearance remain unknown and likely involve multiple pathways. This figure lists many of the cell surface receptors that have been proposed as receptors for Lp(a)

Lp(a) clearance from plasma (Cain et al. 2005). The scavenger receptor B-I, best known for its role in regulating HDL cholesterol uptake, has been reported to bind Lp(a) (Yang et al. 2013). The megalin/glycoprotein 330 receptor, a member of the LDL receptor family, has been shown to bind and take up Lp(a) into cells in vitro (Niemeier et al. 1999). The LDL receptor-related protein-1 has been shown to bind Lp(a) weakly in vitro (Reblin et al. 1997) but has been reported to have no effect on Lp(a) clearance in vivo in animal models (Romagnuolo et al. 2017). The plasminogen receptor PlgRKT has been shown to mediate Lp(a) uptake by HepG2 cells (Sharma et al. 2017). It is interesting to note that following uptake by PlgRKT, the apo(a) component of Lp(a) was trafficked to recycling endosomes and subsequently re-secreted into the cellular media. This would help explain the results of some in vivo human studies that had results showing a slower clearance of apo(a) as compared to apoB100 on Lp(a) which could be explained by apo(a) recycling (Jenner et al. 2005; Diffenderfer et al. 2016). Other receptors that have been shown to have no effect on Lp(a) clearance include the VLDL receptor, LDL receptor-related protein-8 (Romagnuolo et al. 2017), the asialoglycoprotein receptor (Cain et al. 2005), and sortilin (Gemin et al. 2018). Variants in *APOE* have been identified as being

determinants of Lp(a) levels (Clarke et al. 2009; Mack et al. 2017; Li et al. 2015). In addition, apoE in plasma has been shown to have a modest impact on Lp(a) clearance (Li et al. 2015; Cain et al. 2005). It is of interest to note a case report of a patient with apoE deficiency who had an Lp(a) level approximately three-fold higher than the upper limit of normal (Mak et al. 2014) which might be expected if apoE is involved in clearance of Lp(a) or its precursors.

Drug Effects on Lp(a) Metabolism

Statins

Statins inhibit cholesterol synthesis and result in the compensatory upregulation of the LDL receptor, leading to increased LDL catabolism and lower LDL-C levels. Statins were originally thought to have no effect on Lp(a) levels (Kostner et al. 1989) but a meta-analysis of several large statin trials concluded that statins increase Lp(a) levels by ~8–20% (Tsimikas et al. 2020a). The mechanism behind the increase in Lp(a) in response to statin treatment was examined in in vitro studies and was shown to be due to effects on *LPA* gene transcription (Tsimikas et al. 2020a).

PCSK9 Inhibitors

PCSK9 inhibitors block the effect of PCSK9 in mediating LDL receptor degradation, thus leading to increased LDL receptor protein and increased clearance of LDL. They reduce LDL-C levels by about 60%. Treatment with PCSK9 inhibitors has been shown to modestly lower Lp(a) levels by ~20% (Ajufo and Rader 2016). This has been shown to be due to enhanced clearance of Lp(a) from plasma (Watts et al. 2020; Reyes-Soffer et al. 2017). While increased LDL receptor numbers could play a role, it is also possible that PCSK9 influences other factors that affect Lp(a) clearance. Patients heterozygous for PCSK9 gain-of-function mutations have been reported to have Lp(a) levels that were two-fold higher than control subjects (Tada et al. 2016), while heterozygous carriers of PCSK9 loss-of-function mutations have been reported to have Lp(a) levels that are 22% lower than those found in control subjects (Mefford et al. 2019). PCSK9 expression has been shown to slow the uptake of Lp(a) by cultured cells expressing the LDL receptor but had no effect on Lp(a) uptake by cells deficient in the LDL receptor (Romagnuolo et al. 2017). The modest effect of PCSK9 inhibition on reducing Lp(a) is in direct contrast to the effect of statins on increasing Lp(a) and this mystery has yet to be resolved.

Niacin

Niacin can modestly reduce Lp(a) levels. The degree of Lp(a) lowering by niacin has been shown to be greater in subjects with smaller apo(a) isoform size that have elevated Lp(a) levels (Artemeva et al. 2015). Ooi and colleagues examined the mechanism by with niacin reduces Lp(a) have shown that niacin reduced apo(a) and Lp(a) associated apoB100 production with no change in the FCR of these components in subjects treated with niacin (1–2 g/day) with background rosuvastatin treatment (Ooi et al. 2015). Croyal et al. also found that niacin (2 g/day) reduced the production rate of apo(a) in hypertriglyceridemic subjects while the also reducing the clearance rate (FCR) to a lesser degree (Croyal et al. 2015). The mechanism by which niacin can influence Lp(a) metabolism is not clear, although variants in the niacin receptor (hydroxyl-carboxylic receptor 2; HCAR2) have been shown to influence the Lp(a) response to niacin (Tuteja et al. 2017) suggesting that the mechanism may lie downstream of HCAR2 receptor signaling. HCAR2 expression is relatively high in white adipose tissue (Jadeja et al. 2019), and activation of HCAR2 on adipocytes leads to inhibition of triglyceride lipolysis within adipose resulting in reduced fatty acid delivery to liver. However, HCAR2 is also expressed to a lesser degree in liver (Jadeja et al. 2019) and therefore it is possible that activation of HCAR2 on hepatocytes by niacin had direct effects that lead to reduced *LPA* transcription.

Inhibitors of apoB Synthesis/Secretion

The antisense oligonucleotide (ASO) to *APOB*, mipomersen, has been reported to reduce Lp(a) levels by approximately 20–28% (Ajufo and Rader 2016; Santos et al. 2015). This drug, which targets the synthesis and production of cellular apoB100 in liver, might be expected to influence Lp(a) production. However, a kinetic study conducted in patients treated with mipomersen found that the primary effect of the drug on Lp(a) metabolism was to increase the clearance of Lp(a) from plasma, although the reason for this is not entirely clear (Nandakumar et al. 2018). Targeting the hepatic production of apoB100 through use of the microsomal triglyceride transfer protein (MTP) inhibitor lomitapide also reduced Lp(a) levels, although to a lesser extent than mipomersen (Rader and Kastelein 2014).

Other Drugs on Lp(a) Metabolism

Another drug class that has been shown to lower circulating Lp(a) levels are inhibitors of cholesteryl ester transfer protein (CETP) which reduce Lp(a) levels from 24% to 40% (Thomas et al. 2017; Nicholls et al. 2016; Hovingh et al. 2015). This was shown to be due to a decrease in the production of Lp(a) (Thomas et al. 2017), although the mechanism behind this decrease is not entirely clear and requires more study.

While Volanesorsen, an ASO that targets *APOC3*, showed no apparent effects of lowering apoC-III on Lp(a) levels (Tardif et al. 2022), it may provide some insights on Lp(a) biology. ApoC-III is an exchangeable apolipoprotein that has been shown to inhibit both lipoprotein lipase mediated hydrolysis of triglyceride as well as inhibit receptor-mediated uptake of VLDL remnant lipoproteins. Treatment with Volanesorsen was reported to reduce the apoC-III content of Lp(a) (Yang et al. 2016). The fact that Lp(a) levels remained unchanged following treatment would suggest that apoC-III does not influence the clearance of Lp(a) from plasma.

Targeting Apo(a) Production to Lower Lp(a)

A direct approach to lowering Lp(a) levels is to target the synthesis and production of apo(a). The antisense oligonucleotide (ASO) pelacarsen targets the apo(a) mRNA to promote degradation and thus reduces the synthesis of apo(a) protein. Treatment of humans with pelacarsen resulted in a dose-dependent decrease in Lp(a) levels of up to 80% (Tsimikas et al. 2020b). Pelacarsen is now being studied in a phase 3 cardiovascular outcome trial to assess the impact of this degree of Lp(a) reduction on cardiovascular events.

Conclusion

Major advances have been made in our understanding of the factors that regulate Lp(a) metabolism since its discovery 60 years ago. The synthesis of apo(a) is largely under genetic control and ultimately determines the production rate and concentration of Lp(a) in plasma. A single receptor that regulates Lp(a) clearance has not been identified to date; the LDL receptor does not appear to play a major physiological role. Other receptors and apoE have been studied and may have modest effects on Lp(a) clearance from plasma. Thus, it is possible that multiple receptors are responsible for the catabolism of Lp(a). Future studies that examine Lp(a) metabolism, particularly when conducted in response to treatment with novel drugs that influence Lp(a) levels, should lead to further advances in our understanding of Lp(a) biology and the factors that regulate its metabolism.

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Chapter 5
Contemporary Aspects of Lp(a) Metabolism and Therapies Based on Tracer Kinetic Studies in Humans

Dick C Chan, Jing Pang, and Gerald F Watts

Bullet Points

- Lipoprotein(a) [Lp(a)] is an inherited and causal risk factor for atherosclerotic cardiovascular disease (ASCVD) and aortic valve stenosis.
- Use of stable isotope tracers and compartmental modelling has provided deeper understanding of the physiology and pathophysiology of Lp(a) metabolism in humans.
- Plasma Lp(a) concentration is predominantly determined by the rate of production of Lp(a) particles, irrespective of apo(a) isoform size and background therapy with statins.
- Niacin and cholesteryl ester transfer protein inhibitors lower plasma Lp(a) concentration by increasing the clearance or catabolism of apo(a).
- ApoB antisense oligonucleotides lower plasma Lp(a) concentration by decreasing hepatic production.
- Proprotein convertase subtilisin kexin type 9 inhibitors can lower plasma Lp(a) concentration by a dual mode of action involving both increased clearance and decreased production of apo(a),
- Further studies should investigate nucleic acid-based inhibitors for apo(a), angiopoietin-like 3 and apoC-III inhibitors on the metabolism of Lp(a) and other lipoproteins.

D. C Chan · J. Pang
Medical School, University of Western Australia, Perth, WA, Australia
e-mail: dick.chan@uwa.edu.au; jing.pang@uwa.edu.au

G. F Watts (✉)
Medical School, University of Western Australia, Perth, WA, Australia

Lipid Disorders Clinic, Department of Cardiology and Internal Medicine, Royal Perth Hospital, Perth, WA, Australia
e-mail: gerald.watts@uwa.edu.au

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_5

Introduction

Lipoprotein(a) [Lp(a)] is one of the most important genetically determined risk factors for atherosclerotic cardiovascular disease (ASCVD) and aortic valve stenosis (Nordestgaard and Langsted 2016; Saleheen et al. 2017; Tsimikas et al. 2018; Cegla et al. 2009; Arsenault and Kamstrup 2022; Reyes-Soffer et al. 2022). Large clinical trials have consistently shown that patients with elevated Lp(a), even when treated with statins, are at an increased risk of ASCVD (Khera et al. 2014; Nicholls et al. 2010). The metabolic pathways governing the metabolism of Lp(a) have been extensively studied in cellular and animal model systems (McCormick and Schneider 2019; Boffa and Koschinsky 2022; Chemello et al. 2022a). However, only scare information is available on the metabolism of this lipoprotein in humans. Use of stable isotopically labelled tracers and compartmental modelling has greatly enhanced our understanding of Lp(a) metabolism (Chan et al. 2004; Barrett et al. 2006). In the present chapter, we review use of these techniques and its contribution to key knowledge of the physiology and pathophysiology of Lp(a) metabolism in humans. We focus on subjects with elevated Lp(a) and the mode of action of lipid-regulating agents.

Structure and Genetics of Lipoprotein(a) in Brief

Lp(a) is composed of one molecule of a highly polymorphic apolipoprotein(a) [apo(a)] particle covalently linked to one molecule of a low-density lipoprotein (LDL)-like particle containing apoB-100 by a single disulphide bond (Schmidt et al. 2016). Apo(a) is composed of 10 types of kringle 4 (KIV) domains related to plasminogen kringle 4, followed by a KV domain and an inactive protease-like domain. KIV_2 exists in variable numbers (from 3 to >30), which gives rise to Lp(a) isoform size heterogeneity (Marcovina et al. 1996; Kronenberg and Utermann 2013).

The gene encoding apo(a), *LPA*, is located on the long arm of chromosome 6 at 6q2.6–2.7, adjacent to the human plasminogen gene. While the control of *LPA* expression is at present not well understood, certain factors, such as estrogen, hepatocyte nuclear factor 4α, interleukin-6 and tumour necrosis factor alpha, influence the expression of *LPA* (Kronenberg and Utermann 2013). Plasma Lp(a) concentration is largely controlled by the *LPA* gene locus. Up to 90% of its variation in Lp(a) concentration is attributable to genetic factors (Lamon-Fava et al. 1991; Austin et al. 1992; Boerwinkle et al. 1992), with approximately 30–70% explained by a variable number of KIV_2 repeats in the *LPA* gene (Kronenberg and Utermann 2013). The unexplained genetic variance in Lp(a) concentration can be contributed by other genetic factors outside KIV_2 repeat variation. Several single nucleotide polymorphisms (SNPs) in the *LPA* gene, such as rs3798220 (CT/CC) and rs10455872 (AG/

GG), are strongly associated with an elevated Lp(a) concentration (Clarke et al. 2009). Genome-wide association studies (GWAS) have also identified many common genetic variants of small effect which can aggregately influence Lp(a) concentration (Coassin and Kronenberg 2022). Accordingly, a polygenic risk score for predicting Lp(a) concentration has recently been reported, explaining approximately 60–70% of the variance in Lp(a) levels in the EPIC-Norfolk and UK Biobank cohorts (Wu et al. 2021). *APOE* gene is one of the most important genetic factors modulating Lp(a) concentrations (Li et al. 2015; Zekavat et al. 2018; Mack et al. 2017; Chemello et al. 2022b); the ε2 allele is associated with reduced Lp(a) concentrations, whereas the ε4 allele is associated with increased Lp(a) concentrations compared with the ε3 allele (Moriarty et al. 2017). Several physiological states, such as kidney, thyroid and liver disease, and ancestry, also contribute to the variability in Lp(a) concentration (Enkhmaa and Berglund 2022).

Stable Isotopic Tracer Methodologies

Plasma Lp(a) concentration in the circulation is determined by a balance between the rates of production and catabolism of Lp(a) particles. Stable isotope tracer studies using endogenous labelling of apolipoproteins with amino acid precursor molecules (isotopomers) and mathematical modelling have been employed to study Lp(a) kinetics (Barrett et al. 2006). This approach has provided better understanding of Lp(a) homeostasis and of the pathogenesis of elevated Lp(a), as well as the kinetic effects of statin and newer lipid-regulating agents, such as proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors.

Briefly, stable isotopically labelled amino acids (such as D3-leucine) are administered intravenously as a bolus or primed infusion with serial blood sampling over several days to assess the turnover of apo(a). Enrichment data (tracer/tracee ratio) are generated by gas- or liquid-chromatography mass spectrometry (GCMS or LCMS, respectively) analysis after separation of apo(a) from plasma (Chan et al. 2004). A novel LCMS method for quantification of apo(a) enrichment has recently been established by employing a synthetic peptide (LFLEPTQADIALLK) that targets the proteolytic domain of apo(a) following a standardized sample trypsin digestion procedure (Croyal et al. 2015, 2018). This method is more sensitive and less labour-intensive than the traditional approach based on immunoprecipitation and Western blotting. Enrichment data are then analysed via multicompartmental modelling, from which the fractional turnover rate of apo(a) in the circulation is derived. Fractional catabolic (or clearance) rate (FCR) refers to the fraction of trace lost from a defined plasma pool per day. From these primary kinetic data, together with the corresponding plasma pool size of apo(a), absolute transport rates in the circulation are calculated. We have detailed these methods elsewhere (Chan et al. 2004; Barrett et al. 2006). Figure 5.1 shows a multicompartmental model for the metabolism of Lp(a)-apo(a) and Lp(a)-apoB-100.

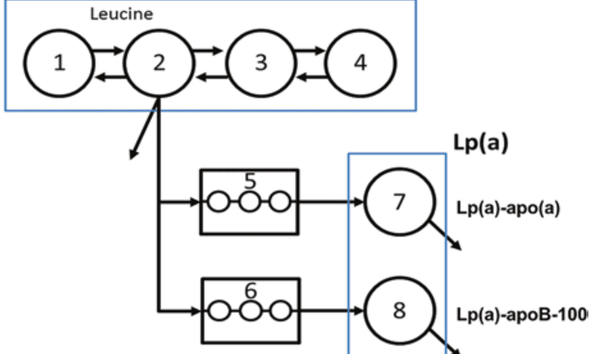

Fig. 5.1 Compartmental model to describe Lp(a)-apo(a) and Lp(a)-apoB-100 tracer kinetics. Plasma leucine kinetics are described by a four-compartment model, which is connected to intrahepatic delay compartments (compartments 5 and 6) that accounts for the synthesis and secretion of Lp(a)-apo(a) and Lp(a)-apoB-100, with compartments 7 and 8 describing the plasma kinetics of Lp(a)-apo(a) and Lp(a)-apoB-100, respectively

Metabolism of Lipoprotein(a)

Synthesis, Assembly and Secretion

Apo(a) is exclusively synthesized by the liver (Schmidt et al. 2016). However, details of the assembly process have not been fully elucidated. The site of Lp(a) assembly may occur in hepatocytes, extracellularly in the space of Disse, or in the circulation (plasma space) (Hoover-Plow and Huang 2013; Youssef et al. 2022). Several pathways for Lp(a) assembly and secretion have been suggested. Apo(a) and apoB are assembled intrahepaticaly, forming an Lp(a) particle which is subsequently secreted into plasma. The Lp(a) particle may also be assembled in the circulation (e.g. on the hepatocyte surface) from its constituent protein; these are then independently secreted from the liver into plasma. There is also uncertainty concerning whether the kinetics in plasma of the two protein components of Lp(a) are coupled, and specifically whether apo(a) is recycled or cleared with apoB-100 as an Lp(a) holoparticle. Using stable isotope tracers and compartmental modelling, we demonstrated that in individuals with a wide range of plasma Lp(a) concentrations, Lp(a)-apoB-100 and Lp(a)-apo(a) have identical isotopic enrichment curves in plasma and similar FCRs (Watts et al. 2018). This finding was confirmed in another kinetic study of statin-treated patients (Ma et al. 2019a) (Fig. 5.2). Hence, these kinetic data generally support that newly synthesized Lp(a)-apoB-100 and Lp(a)-apo(a) are secreted as a holoparticle with tightly coupled apo(a) and apoB100 residence times in the circulation. However, it remains unclear whether the covalent binding of apo(a) to apoB-100 takes place in the liver or in the circulation.

Another outstanding issue concerning the assembly of Lp(a) particles is the extent to which the binding of apo(a) to triglyceride-rich lipoproteins (TRLs) contributes to the formation of Lp(a) particles in the circulation (Nassir et al. 1998;

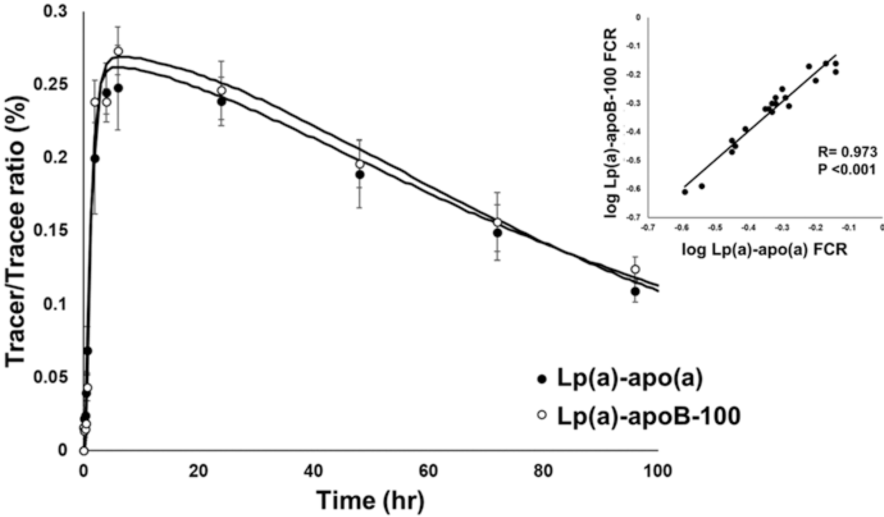

Fig. 5.2 Lp(a)-apo(a) and Lp(a)-apoB tracer-tracee ratio (%) in 20 statin-treated subjects including association of Lp(a)-apo(a) and Lp(a)-apoB-100 fractional catabolic rates (FCR)

Ramos-Cáceres et al. 2022). Earlier radiolabelled kinetic studies suggest that apo(a) is unlikely to be adducted to a triglyceride-rich very low-density lipoprotein (VLDL) as a precursor of Lp(a) in the LDL/HDL density range (Krempler et al. 1980). In contrast, apo(a) can be associated with TRLs, such as chylomicrons and chylomicron remnants, after oral ingestion of a fatty meal (Bersot et al. 1986). This is supported by experimental evidence that the apoB-100-apo(a) complex within Lp(a) particles have a high affinity for TRL particles (Marcoux et al. 1997). A significant proportion of Lp(a) particles can bind non-covalently to TRLs in the hypertriglyceridemic state. Consistent with this, we and others have demonstrated a redistribution of a significant portion of apo(a) protein from Lp(a) to the TRL fraction, particularly in the postprandial state (Cohn et al. 1991; Ying et al. 2022). In a recent study of FH, we found that the impaired postprandial TRL-apo(a) response to a fat load was partially corrected by fish oil supplementation (Ying et al. 2022). The reduction in postprandial TRL-apo(a) with fish oil supplementation in response to the fat load was significantly associated with the corresponding reduction in postprandial triglyceride response. Hence, interaction with TRLs may influence the metabolism or catabolism of Lp(a). The underlying kinetic mechanism remains to be investigated employing stable isotopes and compartmental modelling.

Clearance and Catabolism

It is well established that the liver is the main site of Lp(a) clearance and, to a much lesser extent, the kidney and the arterial wall (McCormick and Schneider 2019). The mechanisms of Lp(a) clearance from the circulation and the catalytic pathways

involved remain uncertain, however. Several cellular receptors have been proposed to mediate the clearance of Lp(a) from the liver. These include LDL receptor and other members of the LDL-receptor family such as VLDL receptor, LDL receptor-related protein 1 (LRP1), megalin/gp330, scavenger receptor class B type 1 (SR-BI) and plasminogen receptor (McCormick and Schneider 2019).

The role of the LDL receptor in Lp(a) clearance remains controversial. Several experimental studies have demonstrated that LDL receptor can facilitate Lp(a) binding and uptake (Havekes et al. 1981; Reblin et al. 1997; Romagnuolo et al. 2015), and in mice overexpressing LDL receptor the clearance of Lp(a) particles is significantly increased (Hofmann et al. 1990). Very few kinetic studies have specifically investigated the metabolism of Lp(a) in patients with LDL receptor defects, such as familial hypercholesterolemia (FH). Using exogenous radiolabelled tracers, Rader et al. found that the clearance of Lp(a) did not differ significantly among homozygous FH patients, heterozygous FH parents and non-FH control subjects (Rader et al. 1995). Using endogenous stable isotope tracers, Croyal et al. reported that the FCRs of Lp(a)-apo(a) were similar in patients with PCSK9 gain-of-function mutations and control subjects (Croyal et al. 2020). Hence, defects in LDL receptor function do not appear to result in delayed clearance of Lp(a). In a study of healthy normolipidemic men, there was no significant association between the FCRs of apo(a) and LDL-apoB-100 (Chan et al. 2019). These kinetic findings suggest under physiological conditions that the LDL receptors may not play a major role in Lp(a) clearance. As discussed later, LDL receptor could play a role in Lp(a) clearance in a supraphysiological condition in which the activity of LDL receptors is markedly upregulated, such as in patients who are treated with a combination of statins and PCSK9 inhibitors (Watts et al. 2018).

Kinetic Determinants of Plasma Lipoprotein(a) Concentrations

Production Rate vs. Fractional Catabolic Rate

In a kinetic study of healthy normolipidemic men with a wide range of plasma Lp(a) concentration, Lp(a) particle concentration was significantly and positively associated with apo(a) production rate (PR) and inversely with apo(a) FCR (Chan et al. 2019). In another study of statin-treated subjects, plasma concentration of apo(a) was significantly and positively associated with apo(a) PR in patients with both normal and elevated Lp(a) concentrations (Ma et al. 2019b). However, there was no significant association between plasma apo(a) concentration and FCR in either of the groups. Hence, these observations reinforce the notion that plasma concentrations of Lp(a) are primarily determined by the rates of production and not clearance, irrespective of background statin use. Accordingly, plasma concentration and PR of apo(a) were significantly higher in statin-treated patients with elevated Lp(a)

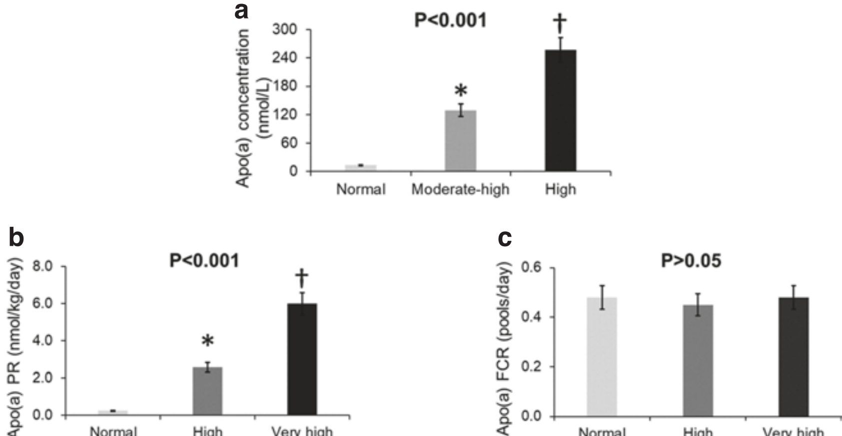

Fig. 5.3 Kinetic parameters of apo(a) in statin-treated subjects with (**a**) normal (<75 nmol/L), (**b**) high (75–145 nmol/L) and (**c**) very high apo(a) concentrations (>145 nmol/L). Data presented as mean ± SEM. *Apo* apolipoprotein, *FCR* fraction catabolic rate, *PR* production rate. $^{*}P < 0.001$ compared with normal apo(a) group. $^{\dagger}P < 0.001$ compared with normal and moderate-high apo(a) group using ANOVA

compared with those with normal Lp(a) (Fig. 5.3a, b). The FCR of apo(a) did not differ significantly between the groups (Fig. 5.3c). This finding suggests that elevated plasma Lp(a) concentration is a consequence of increased hepatic production of Lp(a) particles in patients with elevated Lp(a). In a constant-feeding study of healthy individuals, patients with high Lp(a) had increased apo(a) PR and reduced FCR compared with those without elevated Lp(a) concentration (Jenner et al. 2005). Plasma concentrations of Lp(a) were correlated significantly with both apo(a) PR and negatively with apo(a) FCR. These findings implicate a role of Lp(a) catabolism in determining Lp(a) plasma concentrations in the fed state.

Apo(a) Isoform Size

As discussed earlier, the plasma concentrations of Lp(a) is dependent on genetic variations in the number of KIV_2 repeats (Marcovina and Koschinsky 1999; Kronenberg and Utermann 2013). Experimental data have suggested that the size of the apo(a) transcripts is inversely associated with hepatic *LPA* mRNA concentration (Wade et al. 1991; White et al. 1994) and by implication apo(a) production. Smaller apo(a) isoforms have been shown to have a shorter retention time in the endoplasmic reticulum and probably lesser intracellular apo(a) proteasome degradation, resulting in a more efficiently secretion from hepatocytes (White et al. 1994; Brunner et al. 1996; Lobentanz et al. 1998). On the other hand, Lp(a) with apo(a)

isoforms of different sizes may have different binding affinities for the LDL receptor or other receptors (März et al. 1993). Lp(a) particles with larger isoform size have been shown to be more effectively removed via LDL receptor independent pathways.

In a study of healthy normolipidemic subjects, subjects with smaller apo(a) isoform sizes (≤22 KIV repeats) had significantly higher apo(a) concentration and PR, and lower apo(a) FCR than those with larger sizes (Chan et al. 2019). Plasma apo(a) concentration was significantly associated with apo(a) PR, but not with FCR in subjects with smaller apo(a) isoform size. In contrast, both apo(a) PR and FCR were significantly associated with plasma apo(a) concentrations in subjects with larger isoforms. Similar observations were observed in patients who were on statin (Ma et al. 2019c). Taken together, these findings again suggest that the plasma Lp(a) concentration is predominantly determined by the rate of production of Lp(a) particles, irrespective of apo(a) isoform size and background statin use. Lp(a) particle catabolism may only play a modest role in determining Lp(a) concentration in subjects with larger apo(a) isoform size. These observations also support the clinical use of agents that target the hepatic production and secretion of Lp(a) (Tsimikas 2017).

As discussed earlier, *APOE* genotype can influence the concentration of Lp(a) (Moriarty et al. 2017; Croyal et al. 2020; Chemello et al. 2022a). However, the effect of *APOE* genotype, particularly the presence of apoE2 and apoE4, on Lp(a) concentrations is known to be affected by the size of apo(a) (Klausen et al. 1996; Blanchard et al. 2021). Accordingly, the effect of apoE genotype on the metabolism of Lp(a) in subjects with large and small apo(a) isoform merits further investigation.

Mechanisms of Action of Lipid-Regulating Agents on Lipoprotein(a) Metabolism

A major challenge in managing patients with elevated Lp(a) is a lack of effective and specific treatment for lowering Lp(a) concentrations (Tsimikas 2017; Tsimikas et al. 2018; Reyes-Soffer et al. 2022; Schwartz and Ballantyne 2022). Diet and lifestyle interventions, such as weight loss or physical activity, do not seem to influence Lp(a) concentrations. Lipoprotein apheresis is the only FDA approved treatment for elevated Lp(a). Currently, there are no approved pharmacologic therapies that specifically target Lp(a) concentrations (Cegla et al. 2009; Tsimikas 2017). The kinetic effect of several established and newer therapies, including statins, niacin, PCSK9 inhibitors, cholesteryl ester transfer protein (CETP) inhibitors and apoB antisense oligonucleotides (ASO), on Lp(a) metabolism are discussed below and in Table 5.1, with specific reference to the mechanisms of action.

Table 5.1 Mechanisms of several pharmacological interventions in regulating lipoprotein(a) metabolism

Author (year)	Subjects	Agents	Principal results		
			Concentration	FCR	PR
Watts et al. (2018)	Healthy normolipidaemic men	Atorvastatin	⟺	⟺	⟺
Ooi et al. (2015)	Statin-treated men with type 2 diabetes	Extended-release niacin	↓↓	⟺	↓↓
Croyal et al. (2015)	Non-diabetic, obese men with hypertriglyceridaemia	Extended-release niacin	↓↓	↓↓	↓↓↓
Reyes-Soffer et al. (2017)	Healthy normolipidaemic men and women	Alirocumab	↓	↑	⟺
Watts et al. (2020)	Statin-treated men and women with high Lp(a)	Alirocumab	↓↓	↑↑	⟺
Ying et al. (2022)	Statin-treated men and women with very high Lp(a)	Alirocumab	↓↓↓	↑↑	↓↓
Watts et al. (2018)	Healthy normolipidaemic men	Evolocumab	↓↓	⟺	↓↓
Watts et al. (2018)	Healthy normolipidaemic men	Evolocumab + Atorvastatin	↓↓	↑↑	⟺
Thomas et al. (2017)	Mildly hypercholesterolaemic men and women	Anacetrapib (CETP inhibitor)	↓↓	⟺	↓↓
Nandakumar et al. (2018)	Healthy normolipidaemic men and women	Mipomersen (ApoB ASO)	↓↓	↑↑	⟺

apo apolipoprotein, *ASO* antisense oligonucleotide, *CETP* cholesteryl ester transfer protein, *FCR* fractional catabolic rate, *PR* production rate
↑↑: mild increase; ↓↓: mild decrease; ↓↓↓: marked decrease; ⟺: no change

Statins

The value of statins in lowering LDL-cholesterol is well recognized. Statins competitively inhibit HMG CoA reductase, thereby decreasing cholesterol biosynthesis, reciprocally upregulating hepatic LDL receptors and enhancing clearance of LDL and other apoB-100-containing particles, including TRLs (Ginsberg 2006). Given the structural similarities between LDL and Lp(a), one would speculate that statins could lower Lp(a) concentration by increasing the clearance of Lp(a). However, the effect of statins on Lp(a) levels is conflicting: some studies show a neutral role (Wang et al. 2021; de Boer et al. 2022), while others report a decrease (Takagi and Umemoto 2012) or increase of plasma Lp(a) levels (Tsimikas et al. 2020). It appears that the influence of stains on Lp(a) level may depend on the type of statins; atorvastatin and rosuvastatin increase Lp(a) levels whereas pitavastatin has no impact or

may tend to decrease plasma Lp(a) concentrations (Tsimikas et al. 2020). The statin-induced increase in Lp(a) level is supported by experimental evidence in HepG2 cells showing a higher *LPA* mRNA level in response to atorvastatin (Tsimikas et al. 2020). In a study of healthy normolipidemic subjects, atorvastatin (80 mg daily) did not significantly alter the FCR or PR of apo(a) (Watts et al. 2017). This finding does not support a role of LDL receptor in the regulation of apo(a) FCR under physiological condition. However, it remains unclear whether statin has a potential impact on Lp(a) metabolism in subjects with high Lp(a) concentration. There is also evidence showing that statins increase Lp(a) levels exclusively in patients with a small size apo(a) defined as ≤22 KIV repeats (Yahya et al. 2019). The precise mechanisms of action of this effect on Lp(a) metabolism remain to be investigated.

Niacin

Niacin is one of few agents that can significantly lower plasma Lp(a) concentrations. Experimental data suggest that niacin decreases the expression of *LPA* mRNA (Chennamsetty et al. 2012). This is consistent with a kinetic study showing that niacin lowered Lp(a) concentration by decreasing the production of apo(a) in non-diabetic, obese and hypertriglyceridemic men (Croyal et al. 2015). The lowering of the PR of apo(a) by niacin was confirmed in another postprandial kinetic study in statin-treated patients with type 2 diabetes (Ooi et al. 2015). In this study, extended-release niacin (1–2 g/day) significantly decreased plasma Lp(a) concentration and the production rates of apo(a), with greater treatment effect in individuals with elevated Lp(a) concentration. This is consistent with another study showing that extended-release niacin was more effective in lowering Lp(a) level in subjects with small apo(a) isoform than those with large isoform (Artemeva et al. 2015).

PCSK9 Inhibitors

Inhibition of PCSK9 in combination with statins and/or ezetimibe provides a highly effective approach for lowering LDL-cholesterol concentrations in patients with hypercholesterolemia (Duprez et al. 2020; Ying et al. 2021; Ferri et al. 2020). Monoclonal antibodies (mAbs) targeting PCSK9, such as evolocumab and alirocumab, have been consistently known to significantly lower plasma LDL-cholesterol and the incidence of ASCVD outcomes (Sabatine et al. 2017; Schwartz et al. 2018; Deedwania et al. 2021). PCSK9mAbs can similarly lower plasma Lp(a) concentration. The effectiveness of PCSK9 mAbs in reducing ASCVD events is also found to be most pronounced in patients with high Lp(a) and that the reduction

in Lp(a) could also partly mediate the cardiovascular benefit of PCSK9 mAbs (Bittner et al. 2020; Schwartz et al. 2021).

In a kinetic study of healthy normolipidemic men, evolocumab monotherapy significantly decreased plasma Lp(a) concentration chiefly by reducing the PR of apo(a) with no effect on the corresponding FCR (Watts et al. 2018). This effect is consistent with a tracer study conducted in non-human primates in which alirocumab decreased the PR of apo(a) (Croyal et al. 2018). The mechanistic effect of evolocumab may involve reduced hepatic production of Lp(a) by decreasing the assembly of Lp(a) particles through the reduction of apo(a) binding with LDL on the surface of hepatocytes (Lambert et al. 2017). This speculation is supported by in vitro studies showing that PCSK9 induces Lp(a) intracellular assembly and secretion, whereas PCSK9 mAbs reduce the extracellular release of Lp(a) (Villard et al. 2016).

However, as combination therapy with high-dose atorvastatin, evolocumab reduced the plasma concentration of Lp(a) chiefly by a significant increase in the FCR of apo(a) (Watts et al. 2018). The PR of Lp(a) was not significantly altered with the combination. Similar results were also found in another kinetic study in healthy individuals receiving alirocumab treatment (Reyes-Soffer et al. 2017). However, the increase in apo(a) FCR in the latter study was not statistically significant, probably owing to greater variability in study subject characteristics (e.g. mixed race and gender). The mechanistic effect of evolocumab in combination with atorvastatin may involve supraphysiological upregulation of the activity of LDL receptors and decreased competition of Lp(a) with very low concentrations of LDLs for clearance by these receptors. This mechanism suggests that the LDL receptor likely plays a significant role in mediating Lp(a) clearance only when its expression is markedly upregulated and when LDL plasma levels are substantially lowered, allowing decreased competition between LDL and Lp(a) for receptor-mediated uptake in the liver.

The mechanism of action of PCSK9 inhibition has recently been studied in statin-treated patients with high Lp(a). Using stable isotopes, PCSK9 inhibition with alirocumab-lowered plasma Lp(a) concentration by increasing apo(a) FCR in patients with elevated Lp(a) receiving maximally tolerated statin therapy (Watts et al. 2020). However, in patients with very high-Lp(a) concentration, alirocumab significantly lowered plasma Lp(a) concentration by a dual mode of action involving both increased clearance and decreased production of apo(a) (Ying et al. 2022). Taken together, the mechanistic action of PCSK9 mAbs on the PR and FCR of apo(a) appears to be dependent on background statin use and Lp(a) concentration at baseline.

Unlike evolocumab or alirocumab, small interfering RNA on PCSK9 mRNA transcript (e.g. Inclisiran) is a new approach to targeting PCSK9 intracellularly (German and Shapiro 2020; Smith and White 2022). This novel agent was shown to inhibit hepatic synthesis of the PCSK9 protein, and lower apoB-100-containing lipoproteins, including Lp(a) (Ray et al. 2020; Raal et al. 2020). This implies that the effect of PCSK9 inhibition on Lp(a) is irrespective of mode of inhibition of

PCSK9 (intracellular or extracellular. However, the mechanisms of action of inclisiran on Lp(a) metabolism remain to be elucidated.

CETP Inhibitors

CETP plays an important role in lipoprotein metabolism, primarily by its ability to facilitate transfer of esterified cholesterol from high-density lipoproteins (HDL) to apoB-containing lipoproteins (Tall 1993). Treatment with CETP inhibitors, either alone or in combination with statin, can lower Lp(a) concentrations up to 30% (Schmidt et al. 2021). In a kinetic study of patients with hypercholesterolaemia (Thomas et al. 2017), CETP inhibition with anacetrapib lowered Lp(a) concentration by reducing the PR of apo(a) with no effect on the corresponding FCR. However, there is no clear explanation for the reduction in apo(a) PR with anacetrapib which merits further investigation. Despite these metabolic changes, CETP inhibitors did overall not have clinically meaningful effects in large clinical trials. While several CETP inhibitors, including torcetrapid, evacetrapid, dalcetrapid and anacetrapid, have fallen after disappointing clinical trial outcomes (Berberich et al. 2017; Schwartz et al. 2012; Lincoff et al. 2017; Schmidt et al. 2021), two clinical trials with a newer CETP inhibitor obicetrapib (TA-8995; 10 mg) has been shown to increase HDL-cholesterol by 160%, and reduce LDL-cholesterol, apoB and Lp(a) levels approximately by 50–60%, 30–50% and 25–50%, respectively, in patients treated with atorvastatin or rosuvastatin (Hovingh et al. 2015; Ray 2022). The mechanisms of action of this agent on Lp(a) and other lipoproteins merit investigation.

ApoB Antisense Oligonucleotides

Mipomersen is an antisense oligonucleotide (ASO) directed to liver mRNA of apoB that inhibits apoB synthesis (Parham and Goldberg 2019). Accordingly, mipomersen has been shown to significantly lower plasma concentrations of apoB-containing lipoproteins including LDL and Lp(a). In a kinetic study of healthy volunteers, treatment with mipomersen caused a significant decrease of plasma Lp(a) levels that was associated with a significant increase in the FCR of Lp(a), with no effect on corresponding apo(a) PR (Nandakumar et al. 2018). These results were unexpected because inhibition of apoB synthesis with mipomersen would reduce the availability of apoB100 substrate for the assembly of hepatic apoB with apo(a) to form an Lp(a). It is noteworthy that mipomersen also did not reduce VLDL apoB secretion in the same subjects studied (Reyes-Soffer et al. 2016). These observations appear to support the presence of spare apoB pool in the liver that would be utilized for the assembly of Lp(a) in order to maintain hepatic homeostasis for apoB. However, this speculation requires further investigation. In the same study, the increase in Lp(a) FCR observed was similar to the 30% increase in the FCR of LDL apoB100,

supporting a role for the LDL receptors or related receptors in the clearance of Lp(a) particles.

Other Therapies

Lipoprotein apheresis effectively lowers Lp(a) and LDL levels by approximately 60–70%. Kinetic studies, using stable isotope methods, have shown inconsistent findings when comparing Lp(a) and LDL FCRs in patients undergoing apheresis (Parhofer et al. 1999; Armstrong et al. 1989; Kroon et al. 2000). In studying the rebound of Lp(a) and LDL particle concentration following lipoprotein apheresis (Ma et al. 2019c), the clearance of Lp(a) is significantly slower than that of LDL-apoB in patients with elevated Lp(a) and ASCVD. These findings suggest that the clearance pathways for Lp(a) differ from those of LDL-apoB.

Selective thyroid hormone receptor (THR) agonists (such as Resmetirom) can effectively lower plasma Lp(a) concentrations (Hovingh et al. 2022). Activation of THR has been shown to increase LDL receptor expression, resulting in reduced circulating LDL particles (Erion et al. 2007). Whether the lowering effect of Lp(a) is mediated by upregulating the activity of LDL receptor remains unclear and merits further investigation.

Administration of aspirin has been shown to lower Lp(a) levels in patients with high-Lp(a) concentrations irrespectively of apo(a) isoform size (Akaike et al. 2002). This observation is supported by experimental data in HepG2 cells that aspirin reduced Lp(a) production in H2G cell via the reduction of apo(a) gene transcriptional activity with suppression of apo(a) mRNA expression (Kagawa et al. 1999). However, no kinetic studies have yet specified investigated the effect of aspirin on Lp(a) metabolism in humans.

Lomitapide is a small molecule that inhibits lipid transfer by direct binding to microsomal triglyceride transfer protein (MTP) in the liver and intestine (Berberich and Hegele 2017). By inhibiting MTP in hepatocytes and enterocyte, lomitapide reduces VLDL assembly and secretion, and lowers plasma levels of all apoB-1containing lipoproteins, including VLDL, LDL and Lp(a) independent of LDL receptor (Cuchel and Rader 2013; Harada-Shiba et al. 2017). Accordingly, lomitapide is specifically approved for lowering LDL-cholesterol in homozygous FH (Berberich et al. 2017). Kinetic studies showed a marked reduction in the production of LDL-apoB-100 (Cuchel et al. 2007). Whether lomitapide reduces Lp(a) concentrations by decreasing apo(a) PR remains to be investigated.

Conclusions and Future Perspectives

Lp(a) is associated with an increased risk of ASCVD, even in patients on intensive lipid-lowering therapy. However, elevated Lp(a) remains an under-recognized, under-treated and under-researched condition with an extremely high risk of

ASCVD. This atherogenic disorder has received little attention due to a significant knowledge gap in understanding Lp(a) pathophysiology. Stable isotope tracer methods provide unique information of the dynamics of Lp(a) particles in the circulation. The interferences from these studies are important for understanding the metabolism of Lp(a) and for developing new therapies. Knowledge of the mode of action of therapeutic interventions is also important for informing shared-decision making and improving adherences to therapies. Future research is still needed to understand whole body metabolism of Lp(a), including the stability of the covalent bonding between apo(a) and apoB-100, the potential recycling of apo(a) in the circulation, the possible formation of Lp(a) complexes with TRLs, and the relative roles of hepatic and renal receptors in the clearance of Lp(a) particles. The precise modes of action of CETP inhibitors, apoB ASO and THR agonists on the metabolism of Lp(a), particularly in patients with high Lp(a), also merit further clarification.

While several therapeutic interventions can lower plasma Lp(a) concentrations (Korneva et al. 2021), it is uncertain that it would mitigate the adverse effects of elevated Lp(a) on ASCVD. Nevertheless, some of the cardiovascular benefit of PCSK9 mAbs in clinical outcome trials are known to be mediated by the lowering of Lp(a) independently of the concurrent reduction in LDL cholesterol. More aggressive treatment strategies involve use of multiple lipid-regulating agents to treat elevated Lp(a). This approach harnesses the complementary mechanisms of action of the different agents. Possible combinations include PCSK9 inhibitor with niacin, CETP inhibitor or THR agonist. Inhibiting hepatic apo(a) synthesis with nucleic acid therapeutics has emerged as a potent approach to reduce plasma Lp(a) levels up to 90% which is not affected by *LPA* gene variants and isoform size (Karwatowska-Prokopczuk et al. 2021). The effect of this novel and specific agent on the metabolism of Lp(a) and other apoB-containing lipoproteins warrants investigation. Further studies are required to characterize the mode of action of newer lipid-regulating agents on the metabolism TRLs and Lp(a). These include inhibitors of angiopoietin-like protein 3 (ANGPTL3) and apoC-III (antibodies and/or nucleic acid-based ASO therapies) (Ward et al. 2022).

Conflicts of Interest GFW has received honoraria for lectures and advisory boards or research grants from Amgen, Arrowhead, AstraZeneca, Esperion, Kowa, Novartis, Regeneron and Sanofi. DCC has nothing to declare.

Acknowledgement JP was supported by the National Health and Medical Research Council (HMRC) Investigator Grant.

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Chapter 6
Role of Proprotein Convertase Subtilisin Kexin Type 9 in Lipoprotein(a) Metabolism

Antonio Gallo, Kévin Chemello, Romuald Techer, Ali Jaafar, and Gilles Lambert

PCSK9, a Key Player in Lipoprotein Metabolism

PCSK9 is a serine protease mainly expressed in the liver (Seidah et al. 2014). It is synthesized as a precursor that undergoes autocatalytic intramolecular processing to form a mature enzyme. In 2003, Abifadel and colleagues identified missense mutations in the gene encoding PCSK9 causative of familial hypercholesterolemia (FH) (Abifadel et al. 2003). These mutations were later shown to be gain-of-function mutations. In 2005, a causative association was established between loss-of-function mutations in *PCSK9* and low plasma concentrations of LDL-C, which was accompanied by an astonishing reduction in global coronary heart disease risk (Cohen et al. 2006). Strikingly, studies of individual homozygotes for *PCSK9* loss-of-function mutations demonstrate that a complete or near-complete absence of PCSK9 resulting in very low levels of LDL-C is perfectly compatible with normal human health (Zhao et al. 2006).

Evidence for a direct role for PCSK9 in LDL-C metabolism came initially from a series of studies showing that overexpression of PCSK9 promotes the accumulation of LDL-C in the plasma of control mice but not in that of LDLR-deficient animals (Maxwell and Breslow 2004; Lagace et al. 2006). Mechanistically, the LDLR promotes the cellular uptake of LDL by endocytosis. In the absence of PCSK9, the acidic environment of the endosome promotes the dissociation of the receptor from the LDL particle, and the LDLR is recycled to the cell surface, while LDL is routed to the lysosome for degradation (Nassoury et al. 2007; Qian et al. 2007). PCSK9, which is secreted from hepatocytes, binds to the LDLR at the surface of cells. Following endocytosis, in the presence of PCSK9, the LDLR fails to change

A. Gallo · K. Chemello · R. Techer · A. Jaafar · G. Lambert (✉)
Laboratoire Inserm UMR 1188 DéTROI, Université de La Réunion,
Saint-Pierre (Ile de La Réunion), France
e-mail: antonio.gallo@aphp.fr; kevin.chemello@univ-reunion.fr; ali.jaafar@univ-reunion.fr; gilles.lambert@univ-reunion.fr

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_6

conformation in the endosome, which precludes normal recycling of the receptor to the plasma membrane (Fig. 6.1). The LDLR thus traffics to the lysosome and is degraded along with the LDL particle (McNutt et al. 2007; Li et al. 2007). The increase in LDL-C plasma levels induced by PCSK9 directly stems from the ability of PCSK9 to enhance LDLR lysosomal degradation (Lambert et al. 2012).

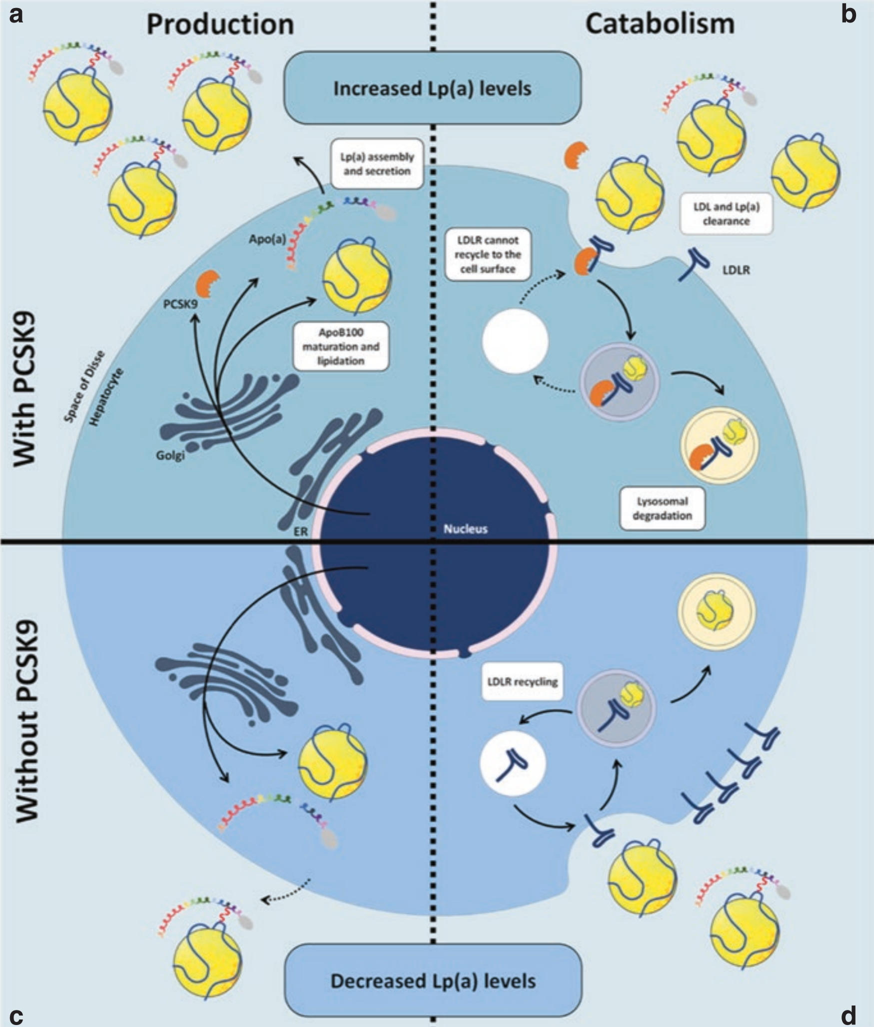

Fig. 6.1 Lipoprotein(a) plasma levels are reduced in the absence or upon pharmacological inhibition of PCSK9—Lp(a) plasma concentrations are primarily determined genetically at the *LPA* locus that chiefly governs the level of apo(a) synthesis and the subsequent rate of Lp(a) particle assembly and secretion by hepatocytes (Panel **a**). PCSK9 targets the LDLR for lysosomal degradation, reducing its abundance at the surface of hepatocytes, and thus lowering the cellular uptake of LDL (Panel **b**). In the absence of PCSK9, the intracellular assembly of Lp(a) may be reduced (Panel **c**), whereas the abundance of LDLR at the cell surface is maximal, allowing optimal LDL uptake by endocytosis without significantly altering Lp(a) clearance (Panel **d**)

PCSK9, an Ideal LDL Cholesterol-Lowering Drug Target

Given the mode of action of PCSK9 as a circulating inhibitor of the LDLR, as well as the healthy profile of individuals with reduced or absent PCSK9 function, PCSK9 rapidly gained status of a very clean drug target to lower LDL-C in humans. Several drug development strategies have been tested to pharmacologically inhibit PCSK9, the most advanced in terms of clinical development being two fully human monoclonal antibodies (mAbs). Large phase III programs with these mAbs have been reported: the FOURIER program with evolocumab (Sabatine et al. 2015) and the ODYSSEY program with alirocumab (Robinson et al. 2015) (Table 6.1). In a

Table 6.1 Lipoprotein (a) reductions by PCSK9 inhibition in Phase III trials

Trial	Patients' number and characteristics	Pharmacological agent and follow-up duration	Lp(a) % median reduction	CV outcome % reduction (hazard ratios)	References
FOURIER	25,096 subjects with established CVD	Evolocumab, 48 weeks	−26.9	Lp(a) upper median: −23% (HR, 0.77 [0.67–0.88]) Lp(a) lower median −7% (HR 0.93 [0.80–1.08])	O'Donoghue et al. (2019)
	4465 subjects from 5 phase 2 and 7 phase 3 trials	Evolocumab, 12 weeks	−25.5	–	Sabatine et al. (2015)
	1359 subjects without CVD with/without background LLT	Evolocumab, 12 weeks	−27.0	–	Stein et al. (2014)
ODYSSEY	18,924 subjects with CVD	Alirocumab, 146 weeks	−23.6	−15% (HR 0.85 [0.78–0.93])	Bittner et al. (2020)
	3499 subjects (placebo-controlled) 1484 (ezetimibe-controlled)	Alirocumab, 84.6 weeks	−25.6 (vs. placebo) −21.4 (vs. ezetimibe)	−12% (HR 0.88 [0.78–0.98])	Ray et al. (2019)
ORION	482 subjects with heterozygous FH	Inclisiran, 77 weeks	−17.2	–	Raal et al. (2020)
	1561 subjects with established CVD 1617 subjects with CVD equivalent	Inclisiran, 77 weeks	−25.6 −18.6	–	Ray et al. (2020)

nutshell, these trials have unequivocally shown that PCSK9 inhibition robustly and safely lowers LDL-C levels regardless of background lipid-lowering therapy and reduces cardiovascular disease (CVD). In addition to mAbs that sequester PCSK9 in circulation and that have been approved by regulating bodies and are now prescribed to patients in many countries, other approaches to PCSK9 inhibition are currently in late-stage clinical development. The small interfering RNA (siRNA) inclisiran that targets PCSK9 hepatic production was approved by the US Food and Drug Administration in December 2021, following reports of positive results of the ORION phase III clinical trial with this drug (Ray et al. 2020).

Statins are the most prescribed lipid-lowering drugs. They enhance *LDLR* gene and protein expression and thereby markedly reduce LDL-C. As mentioned above, PCSK9 inhibitors lower the intracellular degradation of the LDLR, thus increasing the abundance of LDLR at the cell surface and thus reducing LDL-C levels. Whereas statins are neutral or can even elevate the plasma concentrations of Lp(a), PCSK9 inhibitors not only reduce LDL-C (by 50–60% on average) but also concomitantly lower Lp(a) plasma levels by 20–30% (Gaudet et al. 2014; Raal et al. 2016; Lambert et al. 2017). This intriguing observation has led to a flurry of research aimed at investigating the role of PCSK9 in Lp(a) metabolism.

Lipoprotein(a) Plasma Levels Are Chiefly Governed by Production

As mentioned in detail in the previous chapters of this book, Lp(a) is independently and significantly associated with CVD and calcified aortic valve stenosis (Kronenberg and Utermann 2013). Pathophysiological, epidemiological, and genetic studies demonstrate that elevated plasma Lp(a) levels increase the rate of cardiovascular events at any achieved LDL-C level. The major structural difference between Lp(a) and LDL is that Lp(a) has a second large protein, apolipoprotein(a) [apo(a)], bound to the apolipoprotein B100 (apoB100) moiety of an LDL-sized particle by a single disulfide bond (Boffa and Koschinsky 2019). A key feature of Lp(a) is the strong genetic determination of its concentrations, which range over 1000-fold distribution in the population, with the *LPA* gene that encodes apo(a), accounting for more than 90% of this variation. *LPA* alleles contain a variable number of exon pairs encoding plasminogen-like kringle IV (KIV) domains, giving rise to an important size polymorphism of apo(a) isoforms, ranging approximately from 300 to 800 kDa, which corresponds to the presence of one to more than 40 KIV type 2 (KIV_2) domains (Koschinsky et al. 1990). Apo(a) is exclusively synthesized in hepatocytes and undergoes post-translational modifications in the ER (Kraft et al. 1989). The residence time of apo(a) isoforms in the ER is proportional to their number of KIV_2 domains (Brunner et al. 1996; White et al. 1999). As a result, large apo(a) isoforms are more susceptible to proteasomal degradation, which explains to some extent why circulating plasma Lp(a) levels are on average higher in carriers of short apo(a) isoforms.

Several investigators have tried to elucidate the molecular, cellular, and metabolic pathways governing the production of Lp(a), the contribution of Lp(a) to lipid transport in the plasma, and the catabolic fate of Lp(a). The metabolism of this enigmatic lipoprotein nevertheless remains incompletely understood. Unlike LDL, Lp(a) is not the direct product of very low-density lipoprotein (VLDL) metabolism (Krempler et al. 1979). Lp(a) assembly appears to be a two-step process: (1) apo(a) and apoB associate noncovalently through the interactions between weak lysine binding sites located in apo(a) KIV_7 and KIV_8 domains and lysine residues located on apoB100; (2) a disulfide bond is formed between the only "free" cysteine of apo(a) located in its KIV_9 domain and a cysteine located in the C-terminal domain of apoB100 (Gabel and Koschinsky 1998; Youssef et al. 2022). Most in vitro and in vivo kinetic studies suggest that the noncovalent association between apoB100 and apo(a) takes place within hepatocytes, whereas their covalent attachment which is enzyme-catalyzed occurs extracellularly (Youssef et al. 2022). As referred to above, the plasma concentrations of Lp(a) have a strong heritable component related to genetic variations in the number of KIV_2 repeats, with epidemiological studies consistently demonstrating an inverse association between the size of apo(a) and plasma Lp(a) concentrations (Kronenberg and Utermann 2013). Apo(a) production rate and apo(a) isoform size, but not apo(a) fractional catabolic rate (FCR), were shown to be significant predictors of plasma Lp(a) concentrations (Watts et al. 2018). In addition, patients with elevated Lp(a) concentrations have smaller apo(a) isoform sizes and higher apo(a) production rates than patients with normal Lp(a) concentration, the FCR of Lp(a)-apo(a) not differing significantly between these groups of patients. These observations clearly support that plasma concentrations of Lp(a) are primarily determined by the rates of production and not clearance.

The PCSK9-LDLR-Lp(a) Axis

The mechanisms of Lp(a) clearance from the blood and the catalytic pathways involved remain highly uncertain. It is well established that the liver is the major site of Lp(a) clearance followed to a much lower extent by the kidney (Kronenberg 2014). Multiple pathways for Lp(a) clearance have been proposed (McCormick and Schneider 2019). For instance, the scavenger receptor BI (SR-BI) has been shown to promote the selective uptake of Lp(a) cholesterol esters in cells and in SR-BI transgenic mice (Yang et al. 2013). Given the strong homology between apo(a) and plasminogen, the role of plasminogen receptors in mediating Lp(a) clearance has been evaluated (Sharma et al. 2017). One of them, the plasminogen receptor presenting a C-terminal lysine ($PLGR_{KT}$), was shown to mediate the cellular uptake of Lp(a) by human hepatoma cells and primary human fibroblasts. This study also showed that the LDL component of Lp(a) undergoes lysosomal degradation, whereas apo(a) traffics through recycling endosomes and is re-secreted. Several members of the LDLR family of receptors have also been proposed to mediate

whole Lp(a) particle cellular uptake. Thus, the VLDL receptor binds apo(a) and allows the internalization and subsequent degradation of Lp(a) in macrophages (Argraves et al. 1997). The LDLR-related protein 1 (LPR1) and megalin/gp330 (known as LRP2) also play a role in Lp(a) binding (Reblin et al. 1997; Niemeier et al. 1999) cellular uptake and degradation in vitro. LRP8 (formerly known as the apoB,E receptor) is also able to bind Lp(a) at the plasma membrane (Steyrer and Kostner 1990), but it remains to be seen whether this promotes Lp(a) particle cellular uptake and degradation. The cellular uptake of Lp(a) was, however, recently shown to be unaffected in HepG2 hepatoma cells overexpressing either the VLDLR, LRP1, or LRP8 (Romagnuolo et al. 2017). Given the important structural similarities between LDL and Lp(a), and the Lp(a) lowering effects of PCSK9 inhibitors, the LDLR has received the most attention as a candidate receptor for Lp(a) over the past decades.

The initial reports showed that Lp(a) can bind to the LDLR with a lower affinity than LDL (Snyder et al. 1992). It has also been proposed that Lp(a) could associate with LDL and undergo LDLR-mediated clearance by a hitchhiking process (Hofer et al. 1997). In HepG2 and primary human fibroblasts, PCSK9 was shown to reduce the binding and the cellular uptake of Lp(a) via the LDLR (Raal et al. 2016; Romagnuolo et al. 2015). These results were confirmed in HuH7 hepatoma cells and in primary mouse hepatocytes (Romagnuolo et al. 2017). In contrast, other studies found no significant role for the LDLR in mediating Lp(a) cellular uptake in primary human hepatocytes, but also in fibroblasts and HepG2 cells (Sharma et al. 2017; Villard et al. 2016). Neither did they find any significant difference in Lp(a) cellular uptake in primary lymphocytes isolated from normolipemic individuals and patients with homozygous FH who totally lack LDLR function (Chemello et al. 2020). Noteworthy, LDLR expression in human primary lymphocytes positively and significantly correlates with individuals' LDL-C, but not with Lp(a) plasma concentrations (Thedrez et al. 2018).

Studies conducted in mice, rabbits, or nonhuman primates have also yielded opposite conclusions regarding the role of the LDLR and the effects of PCSK9 inhibitors on Lp(a) catabolism. Thus, compared with wild-type animals, mice overexpressing the LDLR display accelerated Lp(a) plasma clearance (Hofmann et al. 1990), but LDLR knockout mice have similar Lp(a) clearance than wild-type animals (Cain et al. 2005). Furthermore, the catabolism of Lp(a) in rabbits is slower than that of LDL, suggesting that Lp(a) uptake is not fully dependent on the LDLR and may be mediated by other mechanisms (Liu et al. 1993). In addition, alirocumab did not alter the catabolic rate of Lp(a) but was found to enhance Lp(a) production in nonhuman primates (Croyal et al. 2018). Likewise, alirocumab had no effect on the hepatic capture of Lp(a) in liver-humanized mice (Chemello et al. 2020).

Studies of FH and non-FH siblings with identical apo(a) isoforms have clearly demonstrated that Lp(a) is approximately twice higher in FH patients than in nonaffected family members (Lingenhel et al. 1998). Homozygous FH subjects with two nonfunctional *LDLR* alleles also display twofold higher Lp(a) levels than their

heterozygote relatives (Kraft et al. 2000). Likewise, familial ligand-defective apoB100 patients (FDB) have higher Lp(a) than non-FDB family members (van der Hoek et al. 1997), and *PCSK9* gain-of-function mutation carriers also similarly display higher Lp(a) than non-FH controls (Tada et al. 2016). The fact that Lp(a) is higher in FH patients has recently been challenged by two independent studies. In 46,200 individuals from the Copenhagen General Population Study in whom Lp(a) was measured, mean Lp(a) concentrations were 23 mg/dL in individuals unlikely to have FH, 32 mg/dL in subjects with possible FH, and 35 mg/dL in those with probable or definite FH, based on the Dutch Lipid Clinic Network diagnostic criteria (Langsted et al. 2016). However, after adjusting LDL-C levels for Lp(a) cholesterol to more accurately assess the FH status, those values were similar at 24, 22, and 21 mg/dL, respectively. Similar observations were made in the British Columbia FH cohort (Trinder et al. 2020). In that cohort, elevated Lp(a) levels in FH were linked to a twofold higher prevalence of a specific single nucleotide polymorphism (rs10455872) on the *LPA* gene associated with an average of 64 mg/dL increase in circulating Lp(a) levels (Trinder et al. 2020) compared with reference populations, suggesting an ascertainment bias in the association between FH and elevated Lp(a). The authors further investigated this possibility using whole-exome sequencing by identifying 221 "true" FH patients (i.e., with pathogenic mutations on the *LDLR*, *APOB*, or *PCSK9* genes) out of 37,486 individuals in the UK Biobank, without prior knowledge of their clinical history. As anticipated, these 221 individuals had significantly higher LDL-C plasma levels than the 37,265 non-FH individuals, but both groups displayed similar circulating Lp(a) concentrations (Trinder et al. 2020). These novel insights cast a doubt on the consensus that Lp(a) is genuinely elevated in FH.

Lipoprotein kinetic studies conducted in humans also provide discrepant conclusions regarding the role of the LDLR and the effects of PCSK9 inhibitors on Lp(a) catabolism. For instance, Lp(a) FCR was similar in control individuals and in homozygous FH patients totally lacking LDLR function (Rader et al. 1995). In contrast, the PCSK9 inhibitor alirocumab was shown to increase (albeit not significantly) the FCR of Lp(a) in one study (Reyes-Soffer et al. 2017), whereas the PCSK9 inhibitor evolocumab in monotherapy did not alter Lp(a) FCR (Watts et al. 2018). In patients with high Lp(a) treated with a high potency statin, alirocumab lowered Lp(a) concentrations by increasing the FCR of Lp(a), but also decreased the particle secretion in those with higher pretreatment Lp(a) concentrations (Watts et al. 2020). There is a large discordance in response to PCSK9 inhibitors in terms of LDL-C and Lp(a) lowering (Edmiston et al. 2017; Shapiro et al. 2019; Blanchard et al. 2022), given that at best only weak correlations between these parameters have been reported. For instance, the FOURIER trial shows a correlation coefficient of 0.37 between changes in Lp(a) and changes in LDL-C levels after 48 weeks of treatment with evolocumab (Raal et al. 2016; O'Donoghue et al. 2019). Similar weak correlations were reported for alirocumab (Mahmood et al. 2021; Gaudet et al. 2017). In a real-life setting, there was no significant correlation between the reduction in apo(a) and the reduction in apoB100 specifically present on VLDL/IDL/LDL after four weeks of PCSK9i treatment (Blanchard et al. 2022).

Conclusion

The fact that unlike statins PCSK9 inhibitors reduce Lp(a) has clearly raised interest in deciphering the molecular mechanisms by which this may occur. Despite much effort, there is no consensus at present indicating that the lowering of Lp(a) induced by PCSK9 inhibitors directly results from the reduction in LDLR expression and function, as is the case for LDL. Figure 6.1 summarizes the potential pathways by which PCSK9 (and hence PCSK9 inhibitors) modulates Lp(a) plasma concentrations. For instance, the LDLR may play some role in mediating Lp(a) clearance when its expression is starkly upregulated (e.g., by concomitant use of statins and PCSK9 inhibitors) and when LDL plasma levels are substantially reduced, allowing decreased competition between LDL and Lp(a) for receptor-mediated uptake. In addition to an effect of PCSK9 on Lp(a) plasma clearance, the latest in vitro evidence points toward a direct role for PCSK9 in enhancing Lp(a) production, assembly, and subsequent secretion from liver cells (Youssef et al. 2022; Villard et al. 2016) (Fig. 6.1). Further exciting research is now needed to extensively explore this fascinating observation in vivo.

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Chapter 7
The Role of Cell Surface Receptors in Lp(a) Catabolism

Lamia Ismail, Déanna Shea, and Sally McCormick

Introduction

Lipoprotein(a) [Lp(a)] is a low-density lipoprotein (LDL)-like molecule that is associated with a significant risk of developing multiple forms of atherosclerotic cardiovascular disease (Nordestgaard et al. 2010; Reyes-Soffer et al. 2022). Lp(a) (Fig. 7.1) is distinguished from LDL by the presence of apolipoprotein(a) [apo(a)], a variably sized plasminogen homologue (McLean et al. 1987), which confers unique properties including the specific binding of oxidised phospholipids (OxPL) (Leibundgut et al. 2013). Apo(a) is synthesised in the liver and requires extensive processing for secretion before it unites with LDL. Lp(a) is cleared principally by the liver with some involvement of the kidney. The pathway for Lp(a) catabolism is complicated as multiple receptor types expressed in both the liver and kidney have been shown to bind and promote Lp(a) uptake. These include members of the lipoprotein, plasminogen, lectin, and scavenger receptor families. There is also some interaction of Lp(a) with toll-like receptors (TLRs) in macrophages during inflammation. In this chapter, we summarise the findings from biochemical and clinical studies documenting the various cell surface receptors that promote Lp(a) catabolism.

Lamia Ismail and Déanna Shea contributed equally with all other contributors.

L. Ismail · D. Shea · S. McCormick (✉)
Department of Biochemistry, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand
e-mail: ismla752@student.otago.ac.nz; deanna.shea@otago.ac.nz; sally.mccormick@otago.ac.nz

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology, https://doi.org/10.1007/978-3-031-24575-6_7

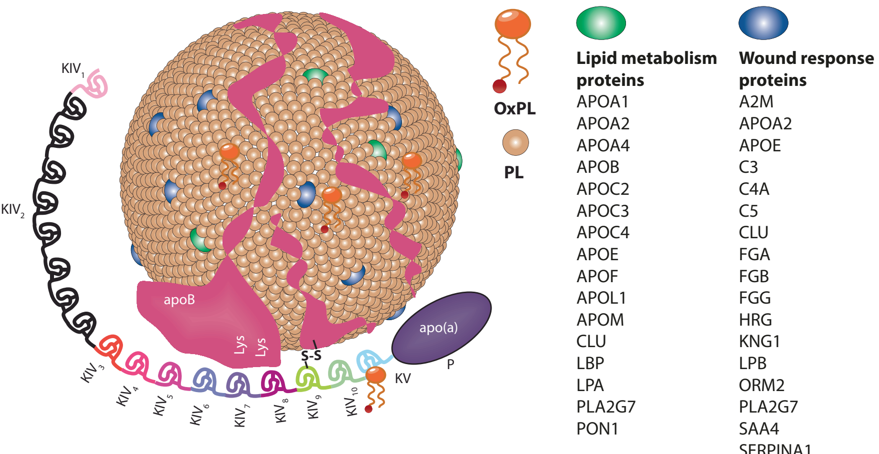

Fig. 7.1 Lp(a) structure. Lp(a) consists of an LDL molecule attached to apo(a) via a disulphide link to apoB. Oxidised phospholipids (OxPL) are found bound to the apo(a) KV domain and contained in the phospholipid (PL) monolayer on the LDL surface. A number of lipid metabolism proteins (green) are associated with the LDL particle, as are many wound-healing proteins (blue), some of which may be bound to apo(a). The proteins are designated by their gene names. (Reproduced from McCormick and Schneider 2019 with permission)

Lp(a) Structure and Assembly

Originally identified as an LDL variant by Kåre Berg (1963), a major difference is that Lp(a) harbours apo(a), a large glycoprotein attached to the LDL surface (Fig. 7.1). Apo(a) is coded for by the *LPA* gene, a plasminogen gene (*PLG*) homologue recently evolved from the duplication of *PLG* kringle IV and V (KIV and KV) (McLean et al. 1987). Further, the *LPA* KIV has 10 different subtypes (KIV1–10) (McLean et al. 1987), with the KIV2 subtype harbouring a copy number variation from 2 to >40 (Kostner et al. 2013) resulting in many different-sized apo(a) isoforms. The apo(a) moiety adds some unique elements to Lp(a) structure since it specifically binds OxPL (Leibundgut et al. 2013) and is associated with various wound response proteins (Von Zychlinski et al. 2011) (Fig. 7.1).

Apo(a) is almost exclusively produced in the liver with small amounts expressed in the testis and brain (Tomlinson et al. 1989). Its synthesis and secretion are regulated by the effects of genetic variation on *LPA* expression and the processing of the apo(a) protein. Recent studies have identified some common single nucleotide variations that are functionally associated with low levels of *LPA* expression (Coassin et al. 2017; Schachtl-Riess et al. 2021). The recognition that *LPA* expression is an important determinant of Lp(a) levels underlies the development of apo(a) antisense oligonucleotides which prevent apo(a) translation in the liver and significantly lower Lp(a) (Tsimikas et al. 2021). With respect to apo(a) processing, each kringle domain requires extensive glycosylation and disulphide cross-linking for proper folding to exit the endoplasmic reticulum; hence, larger isoforms are less efficiently

secreted. This was first shown by studies in primary hepatocytes with different-sized isoforms (White et al. 1997; Brunner et al. 1996) and is supported by recent studies of an apo(a) mutant that displayed defective glycosylation and folding (Morgan et al. 2020). These observations underpin the well-known inverse relationship between Lp(a) levels and isoform size (Kraft et al. 1996).

The location of Lp(a) assembly is not certain but there is significant evidence for an extracellular assembly with LDL (on the surface of hepatocytes or in circulation) after apo(a) secretion (Chiesa et al. 1992; White and Lanford 1994). The process of assembly involves an initial noncovalent binding between apolipoprotein B (apoB) lysine residues exposed on the LDL surface and lysine-binding sites in apo(a) (Gabel and Koschinsky 1998; Becker et al. 2004). This is followed by the formation of a disulphide bond between specific cysteine residues in apo(a) and apoB (Koschinsky et al. 1993; McCormick et al. 1995).

Lp(a) Catabolism

The liver provides the principal route of Lp(a) clearance from the circulation (Cain et al. 2005). However, the presence of apo(a) fragments in the urine of patients with kidney disease (Kostner et al. 1996; Albers et al. 2007) and their reduction after transplantation (Black and Wilcken 1992) also suggest a role for the kidney. In macrophages, it has been documented that Lp(a) binds to and triggers the CD36/TLR2 apoptotic pathway (Seimon et al. 2010).

Unlike LDL, which has a clearly defined uptake pathway via the low-density lipoprotein receptor (LDLR) (Brown and Goldstein 1986) that can be specifically targeted by drugs, i.e. statins and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors, the clearance of Lp(a) is rather more complicated. Many receptors including lipoprotein, plasminogen, lectin and scavenger receptors have been documented as being involved in Lp(a) catabolism (Hoover-Plow and Huang 2013; McCormick and Schneider 2019). Lp(a)'s association with multiple receptors is likely because it has a more complex composition than LDL with more potential ligands (Fig. 7.1); apo(a), apoB and OxPL elements of Lp(a) have been shown to be ligands for the various Lp(a) receptors with apolipoprotein E (apoE), orosomucoid and alpha-2-macroglobulin also reported as possible ligands (McCormick and Schneider 2019).

Liver Receptors

There are multiple receptors that have been shown to interact with Lp(a). Figure 7.2 represents the receptors that have been reported as being involved in Lp(a) uptake that are expressed in the liver and kidney as well as receptors expressed in macrophages which promote cell signalling events from Lp(a). Figure 7.3 shows the gene

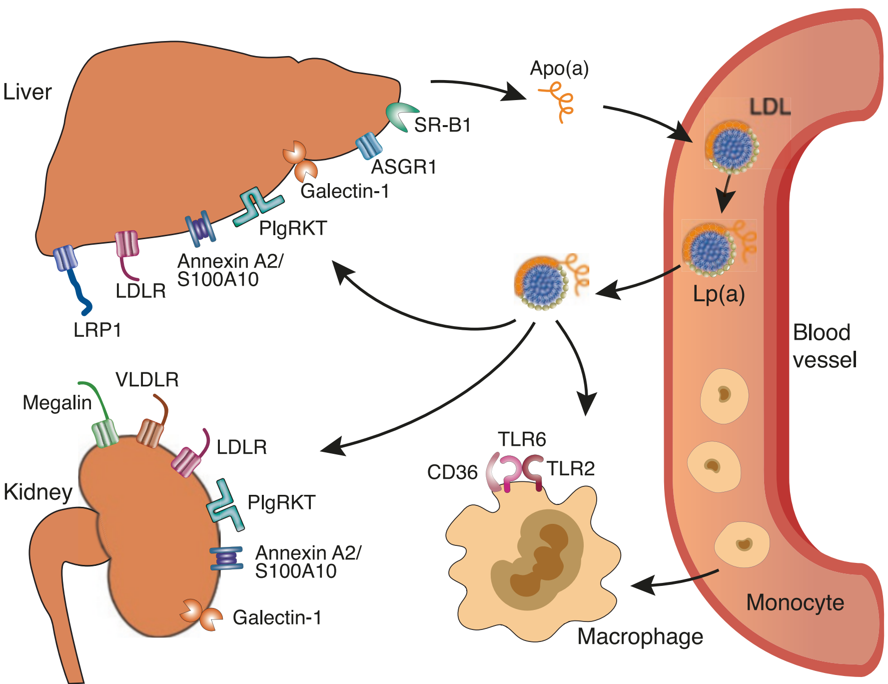

Fig. 7.2 Cell surface receptors for Lp(a). Receptors expressed in the liver and kidney for which there is evidence of a role in binding to and promoting Lp(a) uptake are shown. These include the lipoprotein receptors, LDLR, VLDLR, LRP-1 and megalin; plasminogen receptors, annexin A2, S100A10 and PlgRKT; lectin receptors, galectin-1 and ASGR1; and the scavenger receptor, SR-B1. Also shown are receptors on macrophages which mediate cell signalling events via Lp(a). These include the scavenger receptor; CD36; and toll-like receptors, TLR2 and TLR6

expression profiles of the Lp(a) receptors in both liver and kidney. Most attention has been paid to the LDLR with early cell culture studies showing Lp(a) to bind to fibroblasts via the LDLR (Havekes et al. 1981; Floren et al. 1981) and subsequent hepatocytes studies showing the same (Romagnuolo et al. 2015, 2017). These findings were well supported by a study on fibroblasts from familial hypercholesterolaemia (FH) patients with defective LDL receptors which showed a much-reduced binding to Lp(a) (Krempler et al. 1983). In the same study, kinetic experiments also showed a delayed clearance of Lp(a) in FH subjects (Krempler et al. 1983). However, there are many studies countering these findings including a knockout of the LDLR in mice which showed no difference in Lp(a) clearance compared to wild-type mice (Cain et al. 2005) and kinetics studies indicating no difference in Lp(a) catabolism between FH and non-FH subjects (Rader et al. 1995; Knight 1994). Most importantly, there is the conundrum that statins do not lower Lp(a) and may increase it (Yeang et al. 2016) and that PCSK9 inhibitors, which also upregulate LDLR, have no effect on Lp(a) catabolism (Chemello et al. 2020).

One other LDLR family member that is expressed in the liver (Fig. 7.3) and has been shown to interact with Lp(a) is low-density lipoprotein receptor-related protein

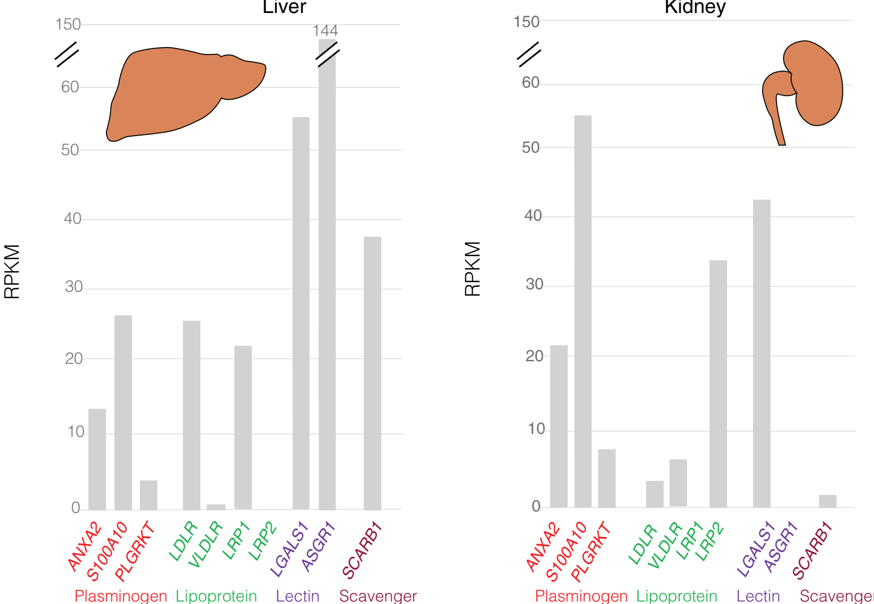

Fig. 7.3 Relative gene expression of Lp(a) receptors. The gene expression data for each of the receptors in the liver and kidney are shown based on reads per kilobase of transcript per million reads mapped (RPKM). Gene expression data were taken from NCBI (https://www.ncbi.nlm.nih.gov/)

1 (LRP1). LRP1 plays an important role in chylomicron remnant catabolism through binding to apoE (Beisiegel et al. 1989). The binding able to be competed by LRP1 to bind Lp(a) (Marz et al. 1993; Reblin et al. 1997) with the binding able to be completed by LDL, plasminogen and alpha-2-macroglobulin (Marz et al. 1993) suggesting multiple ligands.

Not surprisingly, apo(a) interacts with plasminogen receptors on the liver surface. This was first indicated by studies showing that Lp(a) binds to liver cells with the binding able to be blocked by both plasminogen and lysine analogues (lysine dependence is characteristic of plasminogen receptor interactions) (Tam et al. 1996). So far, three members of the plasminogen receptor family have been connected to Lp(a). Lp(a) was found to interact with annexin A2 (Hajjar and Krishnan 1999), an abundant protein associated with membranes and the actin cytoskeleton. Annexin A2 works in conjunction with another plasminogen-binding receptor, S100A10, in the form of a tight heterodimer (Bharadwaj et al. 2021). Interestingly, both proteins are implicated in actin remodelling which is required for macropinocytosis, a clathrin-independent endocytosis pathway known to promote LDL uptake (Kruth et al. 2005) and recently implicated in Lp(a) endocytosis (Siddiqui et al. 2022). Direct evidence for the plasminogen receptors being involved in Lp(a) uptake comes from studies of plasminogen receptor with C-terminal lysine (PlgRKT) (Sharma et al. 2017). Overexpression of PlgRKT in both HepG2 and fibroblast cells

enhanced Lp(a) uptake significantly in both cell lines (Sharma et al. 2017). Furthermore, a much-diminished internalisation of Lp(a) was observed in PlgRKT knockdown HepG2 cells and in PlgRKT−/− fibroblast cells (Sharma et al. 2017). Whether PlgRKT is a *bona fide* receptor for Lp(a) or whether it enhances the surface binding of apo(a) to allow for uptake by other receptors, or via macropinocytosis, is not yet known.

Another receptor highly expressed by the liver (Fig. 7.3) for which evidence is mounting for a role in Lp(a) catabolism is scavenger receptor B1 (SR-B1). Well known for its role in high-density lipoprotein (HDL) metabolism by facilitating selective uptake of cholesterol esters (Acton et al. 1996), it has also been shown to mediate whole particle uptake of very-low-density lipoprotein (VLDL), LDL and HDL (Wang et al. 1998; Zanoni et al. 2018). Evidence for SR-B1's involvement in Lp(a) catabolism has come from transgenic mouse models in which overexpression of SR-B1 significantly increases Lp(a) uptake, and contrariwise, SR-B1 knockout mice show a reduced Lp(a) clearance (Yang et al. 2013). Another study showed that SR-B1 facilitated the uptake of OxPL from Lp(a) in liver cells (Sharma et al. 2015). Interestingly, individuals harbouring a mutation that reduces the ability of SR-B1 to facilitate lipid uptake display both elevated HDL and Lp(a) levels (Yang et al. 2016) suggesting clinical relevance.

One further receptor which is highly expressed in the liver (Fig. 7.3) shown to mediate Lp(a) uptake is the asialoglycoprotein receptor (ASGPR), a lectin receptor that mediates endocytosis of desialylated glycoproteins (Igdoura 2017). Mice lacking the ASGPR showed a much-reduced clearance and degradation of Lp(a) by the liver compared to wild-type mice (Hrzenjak et al. 2003). As apo(a) has a significant content of desialylated O-linked sugars, it is a likely ligand for ASGPR. Indeed, removal of sialic acids from Lp(a) greatly enhanced the clearance rate providing support for an apo(a)/ASGPR interaction (Hrzenjak et al. 2003). Lastly, a lectin receptor, galectin-1, highly expressed in the liver (Fig. 7.3), has been shown to bind to Lp(a) (Chellan et al. 2007).

Kidney Receptors

The kidney expresses many of the same receptors as the liver (Fig. 7.2) including the plasminogen receptors and LDLR. Two other members of the LDLR family expressed in the kidney which have been shown to bind Lp(a) are the VLDL receptor and the megalin receptor (otherwise known as LRP2). The VLDLR has been shown to promote endocytosis and degradation of Lp(a) in fibroblasts with apo(a) mediating the binding (Argraves et al. 1997). Furthermore, mice lacking the VLDLR showed delayed clearance of Lp(a) (Argraves et al. 1997). The megalin receptor is highly expressed in the kidney and plays a role in nutrient uptake via many different ligands (Christensen and Birn 2001). It has been shown to bind to Lp(a) in a yolk sac cell line via its apoB component (Niemeier et al. 1999).

Macrophage Receptors

It is well documented that Lp(a) promotes inflammation via many different signalling pathways through its OxPL content (Tsimikas and Hall 2012; Van der Valk et al. 2016). Macrophages express an array of receptors which bind OxPL and stimulate inflammatory signalling pathways and immune responses (Taylor et al. 2005). These include the TLRs, which often work as co-receptors in conjunction with the CD36 scavenger receptor to sense ligands. The TLR2/TLR6 heterodimer along with CD36 (Fig. 7.2) has been shown to interact with OxPLs on apo(a) to promote inflammation and apoptosis of macrophages (Seimon et al. 2010). Another study indicated that both TLR2 and CD36 were necessary for the ability of Lp(a) to promote IL-8 production from macrophages (Scipione et al. 2015). These studies indicate that the TLRs mediate the signalling promoting properties of Lp(a).

From this array of possible Lp(a) receptors, it is difficult to speculate which receptors might contribute the most to Lp(a) clearance in the physiological setting of the human body. If one considers Lp(a) clearance from a tissue aspect, then evidence suggests that the focus should be on receptors that are highly expressed in the liver. If one considers Lp(a) clearance from a ligand aspect, then the focus might be best placed on receptors that specifically interact with apo(a) since it is a ligand unique to Lp(a).

Summary

The Lp(a) molecule has a complex structure with several of its components known to be ligands for various receptors. Cell culture studies have shown Lp(a) to bind to a diverse range of cell surface receptors on multiple cell types. This situation makes it difficult to pinpoint any one receptor pathway as being important in Lp(a) catabolism and currently precludes targeting Lp(a) catabolism as a route for Lp(a) lowering. Future studies will require a careful teasing out of the roles of the different Lp(a) receptors in the liver and kidney with an emphasis on *in vivo* studies to gauge clinical relevance.DisclosureNothing to disclose for all authors.

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Chapter 8
Physiological Roles and Functions of Lipoprotein(a)

Zaid N. Safiullah, Thorsten Leucker, Steven R. Jones, and Peter P. Toth

Delivery Platform of Oxidized Phospholipids

It has been proposed that Lp(a) has a role in the transport of proinflammatory oxidized phospholipids (OxPL). This hypothesis was driven by observations made by Bergmark et al. who initially developed a method to measure OxPL bound to apoB-100 by using a monoclonal antibody. They demonstrated that plasma OxPL/apoB levels were quantitatively predictive of the presence and extent of angiographically determined CAD, identifying the presence and progression of carotid and femoral atherosclerosis, and predicting CVD events over a 10-year interval. The OxPL/apoB ratio was independent of all known risk factors, except for Lp[a], and, remarkably, in all clinical studies performed at that time, there was an unusually strong correlation of OxPL/apoB with Lp(a) (Bergmark et al. 2008).

These clinical observations suggested that OxPL may be bound to Lp[a] and may thereby contribute to atherosclerotic plaque. In vitro studies demonstrated that not only did OxPL bind to Lp(a), but it was the preferential carrier of OxPL in serum (Bergmark et al. 2008). Notably, when compared to low-density lipoprotein (LDL), Lp(a) bound three times as much OxPL. This led to the proposition that Lp(a)'s physiologic roles may be to preferentially bind and transport OxPLs that are derived from apoptosis and cell death, as occurs during inflammation and oxidative stress, or when OxPLs are mobilized from tissues during iatrogenic plaque rupture during

Z. N. Safiullah
Osler Medical House Staff, The Johns Hopkins Hospital, Baltimore, MD, USA
e-mail: zsafiul1@jhmi.edu

T. Leucker · S. R. Jones · P. P. Toth (✉)
Ciccarone Center for the Prevention of Cardiovascular Disease, Johns Hopkins University School of Medicine, Baltimore, MD, USA
e-mail: tleucke1@jhmi.edu; sjones64@jhmi.edu; Peter.Toth@cghmc.com

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_8

Fig. 8.1 Oxidized phosphatidylcholine-containing phospholipids (Ox-PL). PC1-acyl-2-lyso-sn--glycero-3-phosphatidylcholine. Only sn-2 position composition is shown for all Ox-PL except those forming an ether bond at the sn-1 position. *PAF* platelet-activating factor, *HAz-PC* hexadecyl azelaoyl PC; *13-HODE-PC* 1-palmitoyl-2-(13(*S*)-hydroxy-(9*Z*,11*E*)-octadeca-9,11-dienoyl)-sn-glycero-3-phosphocholine. (Figure and legend reproduced with permission from Lee S, Birukov KG, Romanoski CE, Springstead JR, Lusis AJ, Berliner JA. Role of Phospholipid Oxidation Products in Atherosclerosis. Circulation Research 2012;111:778–799)

PCI or during lesion regression in response to therapeutic interventions. There are many forms of OxPL that can be transported by Lp(a) (Fig. 8.1).

If Lp(a) is the preferential binding partner of oxidized phospholipids, then what are the specific binding domains of OxPLs on Lp(a)? Lp(a) is composed of

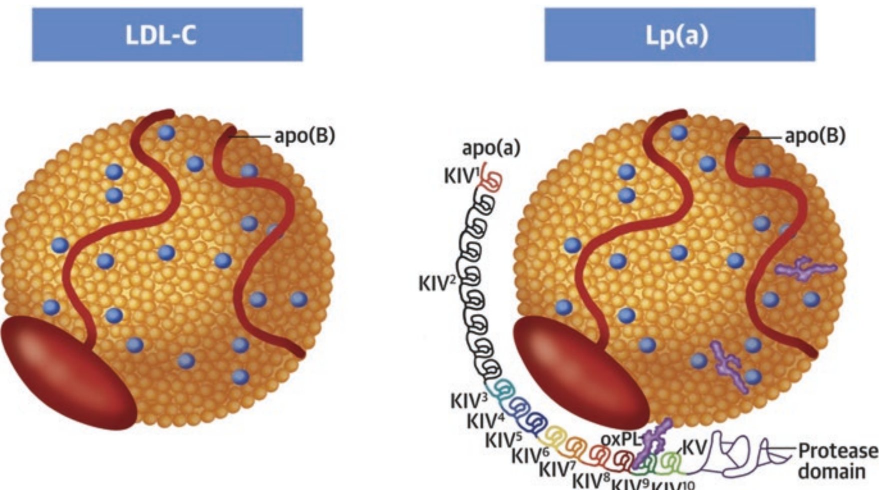

Fig. 8.2 Comparison of LDL and Lp(a) particles. (**Left**) Low-density lipoprotein (LDL) particle; (**right**) lipoprotein(a) [Lp(a)] particle. Apoprotein (apo) B is the scaffolding for lipidation of both lipoprotein species. Lp(a) is an LDL particle that is modified by the covalent addition of apo(a) to apoB. Apo(a) is comprised of a series of kringles (protein loops; kringle IV [1–10] followed by kringle V) and a protease terminus. The number of repeats in kringle IV type 2 are highly variable person to person, genetically determined, and correlate with serum levels of Lp(a) as well as the magnitude of risk for cardiovascular disease exerted by this lipoprotein. *LDL-C* low-density lipoprotein cholesterol. (Figure and legend reproduced with permission from Toth PP. Familial Hypercholesterolemia and Lipoprotein(a): Unraveling the Knot That Binds Them. Journal of the American College of Cardiology 2020;75:2694–2697)

apolipoprotein(a) [apo(a)] covalently bound to apolipoprotein B-100 (apoB) via a single disulfide bond on kringle (K) IV type 9 (KIV9) to a site near the LDL receptor-binding site of apoB (Fig. 8.2). In vitro studies have shown that the amino acid residue 17K Asp 57 of the KIV10 lysine-binding site influences the covalent binding of OxPLs. In vivo studies in mice have also supported the importance of the lysine-binding sites of KIV10 fir OxPL binding (Bergmark et al. 2008). In summary, oxidized phospholipids are proinflammatory and participate in atherogenesis (Chang et al. 2004; Tsimikas et al. 2005). Lp(a) has shown to be the preferential carrier of these molecules when compared to other lipoproteins in vitro and in vivo.

Promoter of Inflammation

Inflammation is a known contributing factor to atherosclerosis development and progression, and to increased cardiovascular disease risk (Fig. 8.3). In this regard, there is ample preclinical and clinical evidence confirming that inflammation plays a pivotal role in multiple steps of atherogenesis by promoting endothelial activation, dysfunction and loss of integrity, failure of endothelial repair capacity, intimal lipid

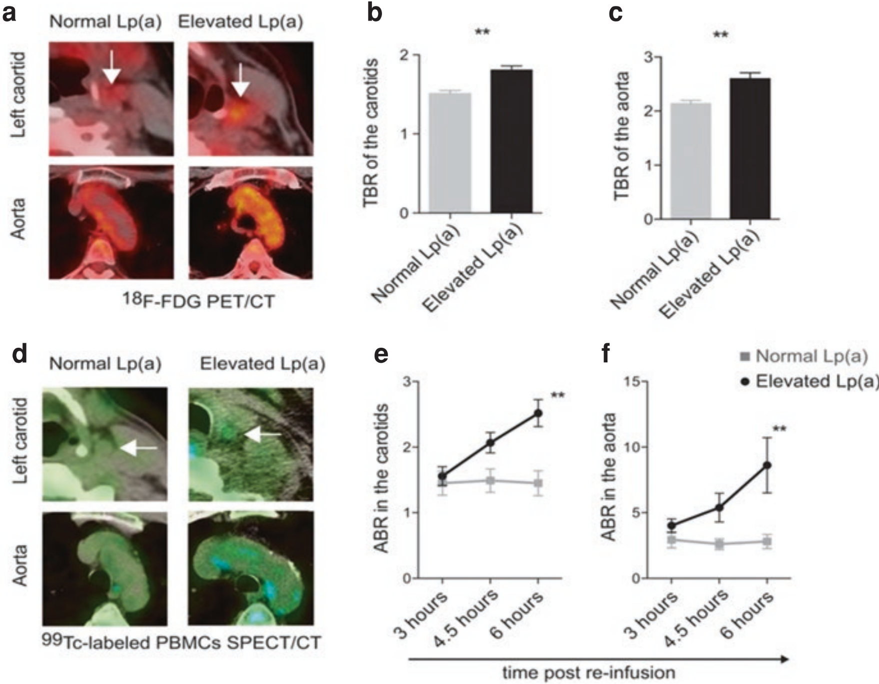

Fig. 8.3 Increased arterial wall inflammation in subjects with elevated lipoprotein(a) [lp(a)]. (**a**) Cross-sectional 18F-fluorodeoxyglucose uptake (18F-FDG) positron emission tomographic/computed tomographic (PET/CT) images demonstrating an increased 18F-FDG uptake (yellow) in the left carotid artery (top, white arrow) and aorta (bottom) in a subject with normal Lp(a) (left) and a subject with elevated Lp(a) (right) quantified as the maximum target-to-background ratio (TBR) in the (**b**) carotid arteries and (**c**) ascending aorta in subjects with elevated Lp(a) ($n = 30$) and normal Lp(a) ($n = 30$). (**d**) Cross-sectional single-photon emission CT (SPECT)/CT images demonstrating increased autologous technetium-99m (99mTc)-labeled peripheral blood mononuclear cell (PBMC) accumulation (blue; at 6 h after infusion), depicted as the arterial wall–to–blood pool ratio (ABR) at the level of (**e**) the carotid arteries and (**f**) ascending aorta in subjects with elevated Lp(a) ($n = 15$) and normal Lp(a) ($n = 15$). $^{**}P < 0.01$. (Figure and legend reproduced with permission from Valk FMvd, Bekkering S, Kroon J et al. Oxidized Phospholipids on Lipoprotein(a) Elicit Arterial Wall Inflammation and an Inflammatory Monocyte Response in Humans. Circulation 2016;134:611–624)

deposition, as well as plaque formation and instability. The detrimental link between inflammation and atherosclerotic CVD risk is further supported by those studies showing that attenuation of inflammation is paralleled by improvement of surrogate indicators of arterial function (e.g., endothelial function, aortic stiffness, ratio of endothelial microparticles to endothelial progenitors) and cardiovascular prognosis (Pirro et al. 2017).

Lp(a) is a well-known acute phase reactant whose production is stimulated by inflammation. Notably, inflammatory response elements are present on the Lp(a) gene. In primary monkey hepatocyte cultures, interleukin-6 (IL-6), IL-4, IL-13, transforming growth factor-β (TGF-β), and tumor necrosis factor-α (TNF-α) can

modulate Lp(a) gene expression. While IL-6 significantly increases plasma Lp(a) levels, other cytokines inhibit Lp(a) synthesis by 50% or more at 10 ng/mL, with the most powerful effect exerted by TGF-β and TNF-α (Ramharack et al. 1998). Although there is sufficient evidence that inflammation may increase plasma Lp(a) levels, data have emerged suggesting that a bidirectional regulatory loop involves Lp(a) and inflammation; thus, Lp(a) may be proinflammatory in most cases, while exerting anti-inflammatory effects in other conditions (Pirro et al. 2017). This pro-inflammatory relationship leads to endothelial dysfunction, fragmentation, detachment, and loss of repair activity, and activates inflammatory signaling cascades.

There is a close link between lipoproteins and inflammation in the arterial wall. Oxidized LDLs (OxLDLs) trigger both directly and indirectly a proinflammatory cascade leading to atherosclerosis development, progression, and complications (Orsó and Schmitz 2017). On entry and trapping by interstitial matrix molecules within the arterial intima, triglyceride-rich lipoprotein degradation by lipoprotein lipase liberates free fatty acids and diacylglycerols, both of which are able to precipitate local inflammation (Orsó and Schmitz 2017). Oxidatively modified lipoproteins can be recognized by toll-like receptors, a type of pattern-recognition receptor that responds against invading microbes, activating early innate recognition, and host inflammatory responses. Also, lipoprotein exposure to reactive oxygen species (superoxide anion, hydroxyl and peroxynitrite radicals) generates diverse oxidized phospholipids (OxPLs) which can contribute to the initiation and the amplification of the inflammatory response. Specifically, OxPLs present on OxLDLs can elicit strong proinflammatory cytokine and chemokine responses from murine macrophages and human monocytes; they can also alter intracellular redox status and directly activate proinflammatory genes, leading to arterial wall inflammation (Pirro et al. 2017; van der Valk et al. 2016).

Lp(a) additionally has a role in initiating and stimulating inflammation, while inhibiting anti-inflammatory pathways (Huang et al. 2014). Lp(a) binds monocyte chemoattractant protein-1 (MCP-1), a chemokine that promotes the binding, rolling, and transmigration of monocytes into the arterial intima (Deshmane et al. 2009). OxPLs have been shown to be major determinants for MCP-1 binding in the vascular endothelium (Wiesner et al. 2013). Lp(a) inhibits the activation of transforming growth factor-beta (TGF-β), a multifunctional and pleiotropic immune regulatory cytokine that participates in peripheral immune tolerance and a negative regulator of inflammation (Kojima et al. 1991). Lp(a) stimulates mRNA and cell surface expression of intercellular adhesion molecule-1 (ICAM-1) in cultured human umbilical vein endothelial cells (HUVECs) (Takami et al. 1998). Lp(a)-induced expression of ICAM-1 in HUVECs appears to be mediated by decreasing active TGF-β availability. Furthermore, Lp(a) promotes monocyte adhesion and trans-endothelial migration by stimulation of MCP-1 and chemokine I-309, which is the principal monocyte chemotactic cytokine produced by T helper cells (Haque et al. 2000). Lp(a) promotes the differentiation of proinflammatory M1-type macrophages, leading to activation of T-helper-1 lymphocytes and natural killer cells. Thus, macrophage expression of interleukin-1 (IL-1), IL8, and TNF-α is stimulated by Lp(a) (Klezovitch et al. 2001). Secretion of these proinflammatory cytokines can

further induce endothelial activation by promoting ICAM-1, vascular cell adhesion molecule-1 (VCAM-1), and E-selectin cell surface expression on endothelial cells. Other than modulating inflammatory cells' activity, Lp(a) stimulates the release of proinflammatory cytokines from vascular endothelial and smooth muscle cells (Klezovitch et al. 2001; Schmitz and Orsó 2015). Finally, apo(a), the distinguishing kringle-containing component of Lp(a), is able to directly elicit a proinflammatory response by inducing nuclear-catenin-mediated cyclooxygenase-2 (COX-2) expression (Pirro et al. 2017; Orsó and Schmitz 2017).

Lp(a) is associated with heightened systemic inflammation (Pirro et al. 2017). In end-stage renal disease patients on hemodialysis, increased Lp(a) levels are associated with systemic inflammation and immune dysregulation. Plasma Lp(a) levels are significantly higher in patients with elevated C-reactive protein (CRP) levels than in those with plasma CRP levels within the normal range. van der Valk et al. (2016) reported that subjects with increased plasma Lp(a) levels show radiologic evidence of arterial inflammation and increased inflammatory cell trafficking to the arterial wall. Intriguingly, these proinflammatory effects are mediated by Lp(a)'s OxPL content. In summary, Lp(a) may activate several pathways linked to local and systemic inflammation (Pirro et al. 2017; Orsó and Schmitz 2017; van der Valk et al. 2016).

There is an abundance of data that describes Lp(a)'s role in stimulating and sustaining inflammation. However, there is also some evidence that Lp(a) also has anti-inflammatory properties. Lp(a)-mediated OxPL scavenging and, in some circumstances oxPL degradation by Lp(a)-lipoprotein-associated phospholipase A_2, may exert anti-inflammatory effects (Tsimikas and Witztum 2008; Kiechl et al. 2007). Furthermore, in two inflammatory models (i.e., thioglycollate-induced peritonitis or $CaCl_2$-induced abdominal aortic aneurysm), apo(a) inhibits neutrophil recruitment by inhibiting cytokine release and reducing entry of neutrophils into the vessel wall (Huang et al. 2014). However, this observation was accompanied with a leukocytosis so a concomitant inflammatory process cannot be excluded.

Impact on Malignancies

Following from its role as a proinflammatory agent, Lp(a) has a complicated role in cancer biology. Observational studies have elucidated varying levels of Lp(a) in different malignancies. Elevations in serum Lp(a) are associated with lung cancer and metastatic breast cancer (Orsó and Schmitz 2017; Lippi et al. 2007). However, Lp(a) levels are found to be mostly decreased in hepatocellular cancer with some variability (Orsó and Schmitz 2017; Gao et al. 2018). Lp(a) serum levels seem to be independent of ovarian cancer, as well as acute lymphoblastic lymphoma. Observationally, it is unclear if there is a singular relationship between Lp(a) concentration and cancer.

Lp(a) staining studies have identified Lp(a) in the tumor vasculature, rather than in the primary parenchyma of the tumor (Lippi et al. 2007; Correc et al. 1990).

Angiogenesis is vital to tumor growth and proliferation. It is thought to be driven by an imbalance in angiogenic and angiostatic factors (Ramanujan et al. 2000). A prototypical example of an angiostatic molecule is angiostatin. Angiostatin is not a novel encoded protein, but rather a degradation product of proteins with kringle domains (Wahl et al. 2005). This domain is responsible for angiostatin's antiangiogenic properties. In vitro experiments have shown that disrupting the tertiary structure of the kringle domain leads to disinhibited angiogenesis (Cao et al. 1996). This kringle domain is found in the PLG domain of Lp(a) (Lippi et al. 2007).

The specific mechanism by which kringle fragments inhibit endothelial cell proliferation remains an issue of ongoing investigation. It is theorized that the antiangiogenic potential of the different kringle domains may depend not only on appropriate protein folding from disulfide linkages but also on the individual primary amino acid sequence. Although the amino acid sequence alignment of the PLG kringle domains shows that kringles I, II, III, and IV display significant sequence homology (48–50% identity) (Lippi et al. 2007), kringle I was identified as the most potent inhibitor for endothelial cell growth. Kringle III exhibited higher inhibitory potency than kringle II and, interestingly, kringle IV was virtually inactive in the suppression of endothelial cell growth (Cao et al. 1996; Dominguez et al. 2001). Since the removal of kringle IV from angiostatin potentiates its inhibitory activity on endothelial cells, it is conceivable that this specific domain may prevent some of the inhibitory effect of kringles I–III. Amino acid sequence alignment reveals that kringle V displays the highest sequence identity with kringle I (57.5%) (Lippi et al. 2007; Cao et al. 1997). Therefore, the high degree of similarity in the primary structure may relate to the potent inhibitory activity of these two kringles on endothelial cell growth. In vitro studies concluded that the order of endothelial cell inhibition may be kringle V > kringle I > kringle III > kringle II > kringle IV (Lippi et al. 2007; Cao et al. 1996, 1997). However, it is unknown if this order of inhibition is seen in vivo.

In addition to the angiogenesis that promotes tumor proliferation, cell adhesion and thrombus formation are processes that also aid in tumor growth (Lippi et al. 2007; Kim et al. 2004). Fibrin deposition following local thrombin generation increases endothelial cell motility and promotes angiogenesis (Staton and Lewis 2005). The apo(a)-dependent inhibition of fibrinolysis might play a role in promoting tumor proliferation.

Evidence from animal models have supported an antineoplastic role of Lp(a) seen in vitro. Experiments have shown that angiostatin can maintain metastases in a dormant state and shrink primary tumors by blocking neovascularization and tumor growth in vivo. Accordingly, several clinical investigations confirmed the potent antineoplastic effect of a recombinant form of apo[a] in the animal model. The retroviral gene transfer of murine colon carcinoma cell line CT26 with LK68 (a recombinant of Lp(a) kringle type IV and V) significantly suppressed tumor growth, as well as progression of micrometastases to macroscopic tumors and peritoneal dissemination (Yu et al. 2004, 2005). Intramuscular administration of virus carrying genes encoding for LK68 gave 60–84% suppression of tumor growth in mice bearing subcutaneously transplanted hepatocellular carcinoma (Lee et al. 2006). Overall,

improved mortality was seen in mice expressing LK68 and bearing human colorectal cancer, lung cancer, or hepatocellular carcinoma (Lippi et al. 2007).

A large prospective cohort study of over 10,000 patients showed that low serum Lp(a) levels correlated with increased mortality from cancer, specifically hepatocellular carcinoma (HCC) (Lippi et al. 2007; Katzke et al. 2017). In HCC, increased Lp(a) has been linked with decreased recurrence after resection. Further, it has utility as a prognostic marker in α-fetoprotein <400 ng/mL level and tumor size <5 cm in subgroups from a small prospective study. Similarly, a retrospective study that compared serum Lp(a) concentration with tumor size in papillary thyroid cancer found a significant negative correlation and concluded that Lp(a) may have a protective effect. Finally, a German cohort study found that increased Lp(a) levels were associated with a total reduction of cancer mortality (Lippi et al. 2007; Katzke et al. 2017).

Alternatively, a retrospective analysis done to investigate the association between Lp(a) levels and clinical pathologic features of prostate cancer showed that higher Lp(a) levels correlated with a high-risk prostate cancer phenotype (Wang and Zhang 2019). These results were similar to a separate German analysis (Katzke et al. 2017). The authors theorized that increased Lp(a) levels may be compensatory to chronic inflammation secondary to aggressive prostate cancer. Furthermore, increased Lp(a) may promote increased cancer cell adhesion, invasion, and metastasis through its role as a competitive inhibitor of plasmin-induced fibrinolysis (Wang and Zhang 2019). The clinical significance of Lp(a) in malignancy requires further investigation. Presently, Lp(a) may be protective in HCC and papillary thyroid cancer (Ma et al. 2021). Conversely, it may be deleterious in prostate cancer. More research is needed to understand the paradoxical underpinnings of these phenotypes.

Lp(a)'s angiostatic properties may make it a novel target for cancer therapeutics. There are preclinical studies that have shown the utility of angiostatin gene therapy in decreasing angiogenesis. Small molecule delivery and gene therapy do not have cytotoxic side effects seen in conventional chemotherapy and immunotherapy. Further research is needed to investigate whether the derived angiostatic mechanisms of Lp(a)'s kringle domain can be developed into cancer therapies (Lippi et al. 2007).

Lp(a) in Thrombosis

Lp(a) has a well-characterized role in thrombogenesis. Biochemically, Lp(a)'s apo(a) moiety has a sequence homology to plasminogen. Plasminogen is a proenzyme of plasmin, which participates in fibrinolysis. Functional studies have illustrated that apo(a) is a competitive inhibitor of pericellular plasminogen activation (Boffa and Koschinsky 2016).

Plasminogen consists of an N-terminal tail domain, five different kringle domains, and a latent trypsin-like protease domain (Forsgren et al. 1987). The kringle domain is common to other proteases involved in hemostasis such as prothrombin, Factor VIII, tissue plasminogen activator (TPA), and urokinase plasminogen

activator (Patthy 1985). The apo(a) subunit consists of ten different types of kringle domains, differing in amino acid sequence, which are most homologous to plasminogen kringle IV (KIV), and a single plasminogen kringle V (KV)-like domain and a protease-like domain (McLean et al. 1987). Of the ten KIV types in apo(a), nine are present in single copy in all apo(a) isoforms (van der Hoek et al. 1993), while KIV type 2 (KIV2) is encoded in a variable number of tandemly repeated copies by the apo(a) gene (LPA), generating a series of different-sized LPA alleles and, hence, apo(a) isoforms in the human population. Known alleles encode as few as 1 and as many as 34 KIV2 repeats, giving rise to apo(a) isoforms containing between 10 and 43 KIV-like domains, and polypeptide molecular masses between 200 and 800 kDa (Boffa and Koschinsky 2016).

Mechanistically, kringles are thought to function in ligand interactions with lysine-containing substrates. Several of the kringles in plasminogen contain lysine-binding sites (LBSs), defined structurally by a hydrophobic trough, lined by two or three key aromatic side chains, which binds the aliphatic backbone of the lysine side chain and that is flanked on either end by a cationic and anionic center (Hoover et al. 1993; McCance et al. 1994). Regarding LBS in plasminogen, the LBS in kringle I has the highest affinity for lysine analogs, followed by KIV and KV (Castellino and McCance 1997). The LBSs in plasminogen have been shown to be important for both lysine-dependent interactions with substrates, such as fibrin and cell-surface receptors, as well as for intramolecular interactions that maintain the closed native conformation of plasminogen (Boffa and Koschinsky 2016; Cockell et al. 1998; Violand et al. 1978).

The KIV types within apo(a) have varying affinities for lysine binding, with KIV10 having a stronger affinity than KIV5–KIV8. The differential in lysine-binding affinity is due to conservative amino acid substitutions (Ye et al. 2001; Rahman et al. 2002). KIV10 is the only kringle domain that interacts with lysine-containing substrates because the LBS in KIV5–KIV8 are masked when bound to apoB-100 in Lp(a). In fact, KIV7–KIV8 have been explicitly shown to participate in noncovalent interactions with specific lysine residues on apoB-100 that precede covalent Lp(a) formation (Boffa and Koschinsky 2016).

In addition to the kringle domain, apo(a) has a protease domain. The protease-like domain in apo(a) is catalytically inactive, despite having an intact Ser-His-Asp catalytic triad (Gabel and Koschinsky 1995). An Arg to Ser substitution at the location analogous to the site on plasminogen that is cleaved by plasminogen activators ensures that an activating cleavage of apo(a) cannot occur (Hajjar et al. 1989). In addition, several other amino acid substitutions relative to plasminogen, as well as a key nine amino acid deletion in apo(a), have been proposed to render the protease-like domain in apo(a) inactive (Boffa and Koschinsky 2016; Gabel and Koschinsky 1995).

Lp(a) lacks protease activity while retaining the ability to bind to lysine-containing substrates; hence, it is possible that Lp(a) may interfere with the functions of plasminogen through molecular mimicry. This has been characterized by several in vitro studies that have shown that Lp(a) interferes with plasminogen activity (Boffa and Koschinsky 2016; Hajjar et al. 1989; Miles et al. 1989; Romagnuolo et al. 2014). The lysine-binding function of plasminogen is crucial to

its fibrinolytic role. Activation of plasminogen by tPA occurs slowly in the absence of a fibrin surface. In the presence of fibrin, a ternary complex is formed that results in efficient production of plasmin (Hoylaerts et al. 1982). Plasminogen binding to fibrin converts the protein from a closed to an open conformation that makes it a better substrate for tPA (Urano et al. 1988). Further, partial degradation of fibrin by plasmin results in the formation of carboxyl terminal lysine residues that mediate positive feedback in the fibrinolytic cascade by promoting: (1) plasminogen binding (Suenson and Petersen 1986), (2) plasmin-mediated conversion of native Glu1-plasminogen to Lys77-plasminogen, which lacks the tail domain and is a better substrate for tPA (Suenson and Thorsen 1988), and (3) binding to plasmin and thus protecting it from consumption by antiplasmin (Boffa and Koschinsky 2016; Wiman and Collen 1978).

The first studies that explored the functional implications of the homology between apo(a) and plasminogen demonstrated the ability of Lp(a) and apo(a) to inhibit binding of plasminogen to cell surface receptors on monocytes and endothelial cells (Hajjar et al. 1989; Miles et al. 1989). Apo(a) was presumed to be a competitive inhibitor of pericellular plasminogen activation. This was confirmed by later experiments (Romagnuolo et al. 2014). It is also thought that this mechanism may contribute to atherogenesis secondary to persistence of mural thrombi or extracellular matrix degradation of the vascular wall (Boffa and Koschinsky 2016).

Regarding the effect of apo(a) on tPA, in vitro studies have demonstrated that apo(a) and Lp(a) are capable of inhibiting tPA-mediated clot lysis and inhibiting tPA-mediated plasminogen activation (Boffa and Koschinsky 2016). Furthermore, apo(a) can inhibit the positive feedback step of plasmin-mediated Glu- to Lys-plasminogen conversion in the context of fibrin. However, when it comes to understanding the mechanistic underpinnings of this observation, the data have been mixed. Some data support that apo(a) directly competes with plasminogen for binding to fibrin. Whereas an alternate mechanism has been postulated that apo(a) forms a quaternary complex with plasminogen, tPA, and fibrin that has a much lower turnover number than the ternary complex lacking apo(a) (Boffa and Koschinsky 2016).

Thus far we have explored the antifibrinolytic mechanisms of Lp(a). Additionally, Lp(a) has well-characterized prothrombotic mechanisms including the promotion of platelet aggregation and tissue factor pathway inhibitor (TFPI) binding (Boffa and Koschinsky 2016). Lp(a) has a dual role in platelet aggregation (Boffa and Koschinsky 2016). Experiments have shown that Lp(a) enhances platelet aggregation and granule release mediated by the thrombin receptor activation peptide SFLLRN. It has also been shown that Lp(a) potentiates arachidonic acid-induced platelet aggregation. Mechanistically, this is mediated by binding of apo(a) to lysine residues on platelet receptors. Alternatively, there is evidence that Lp(a) or apo(a) decreases platelet activation induced by collagen, ADP, or platelet-activating factor. This duality of function may exist as a counterbalance to Lp(a)'s antifibrinolytic activity (Boffa and Koschinsky 2016).

Lp(a) also directly binds tissue factor pathway inhibitor (TFPI). TFPI inhibits the extrinsic coagulation cascade by binding to Factor Xa and then the tissue factor/Factor VIIa complex. Apo(a) binds to TFPI through lysine residues in the carboxyl-terminal portion of TFPI and decreases its fluid phase and cell surface activity,

thereby inhibiting coagulation. Furthermore, the binding of Lp(a) to TFPI may contribute to its atherogenic potential. Immunostaining studies on coronary atherectomy samples have showed TFPI and apo(a) in smooth muscle cell-rich areas of the intima (Boffa and Koschinsky 2016; Caplice et al. 2001).

Lp(a) also interacts with the fibrin clot. The fibrin clot is the final product of primary hemostasis. The clot structure is important in determining both the stability and fibrinolytic capacity of the fibrin clot, which has implications for abnormal thrombolysis. Unfortunately, as compared with the role of Lp(a) in antifibrolytic and prothrombotic, its mechanism of interaction with fibrin is not as well understood.

Observational studies have shown that elevated Lp(a) levels have been associated with an altered fibrin clot structure that is accompanied by reduced fibrin clot permeability and impaired fibrinolysis (Boffa and Koschinsky 2016). This is thought to be one of the etiologies of how Lp(a) contributes to CAD. Furthermore, from a genetics perspective, the LPA gene contains a SNP (rs3798220) that results in an Ile to Met substitution at amino acid 4399 within the protease-like domain of apo(a). The allele encoding Met has been associated with elevated plasma Lp(a) levels, small apo(a) isoform sizes, and increased risk for congenital heart disease (Luke et al. 2007). Caucasians heterozygous for the Ile4399Met variant exhibit elevated Lp(a) levels, increased clot density, and increased clot lysis times, while non-Caucasian carriers showed increased clot permeability and shorter lysis times, with no significant increase in Lp(a) levels. Interestingly, in the Women's Heart Study, individuals heterozygous for the Ile4399Met variant exhibited elevated Lp(a) levels and an increased risk for CAD and benefited more from aspirin therapy than wild-type subjects (Boffa and Koschinsky 2016). This could suggest a prothrombotic role for the Ile4399Met polymorphism.

Most studies that examined the association between Lp(a) and venous thromboembolic events (VTE) are cross-sectional. A meta-analysis of ten studies revealed that among 13,541 subjects, those with a history of deep vein thrombosis were more likely to have elevated Lp(a) (Dentali et al. 2017). Elevated Lp(a) was associated with the presence of VTE at an odds ratio of 1.56 (95% CI 1.36–1.79). There was also a stronger association between Lp(a) and VTE in patients with other predisposing risk factors. A study of 467 patients with first VTE followed up for 1 year by Marcucci et al. found a fivefold increased risk of recurrent VTE for Lp(a) >30 mg/dL (OR 5.1, 95% CI 3.1–8.4), a level of risk similar to that seen in hyperhomocysteinemia and even higher than that for factor V Leiden or the factor II 20210GA polymorphism (Caplice et al. 2001; Dentali et al. 2017; Crowther 2004; Nave and von Eckardstein 2019).

Lp(a) in Diabetes

The mechanism by which Lp(a) influences the development of type 2 diabetes is not well understood. Retrospective cohort studies have shown a negative correlation between Lp(a) and insulin resistance (Mora et al. 2010). In a sample of 607 dyslipidemic patients, Lp(a) correlated inversely with serum triglycerides (TG) levels, TG/

HDL-C ratios, insulin, HOMA-IR, C-peptide, body mass index, and waist circumference (Vaverková et al. 2017). Another study has also illustrated that there is a sharp decrease in Lp(a) levels in the transition from prediabetes to type 2 diabetes (Kaya et al. 2017). Genetic data from Chinese and Danish populations have shown an increased risk of type 2 diabetes in individuals with genetically determined low lipoprotein(a) plasma concentration due to large lipoprotein(a) isoform size related to the number of kringle IV type 2 repeats. Alternatively, a Mendelian randomization analysis showed that genetic variants associated with fasting insulin levels bore no relation to Lp(a) concentration (Mora et al. 2010; Kamstrup and Nordestgaard 2013).

Like retrospective data, large prospective studies have also shown that there is a negative association between Lp(a) and the risk of developing type 2 diabetes (Mora et al. 2010; Schwartz et al. 2021). The negative association between Lp(a) and type 2 diabetes has also been observed to be dependent on the concentration of Lp(a). Lower Lp(a) levels were associated with an increased risk of type 2 diabetes. Similarly, it has been shown that Lp(a) levels are increased in prediabetes compared to normoglycemic controls (Paige et al. 2017). Regarding sex differences, the inverse relationship between Lp(a) and increased glucose levels is observed in men prior to prediabetes. Whereas in women, the inverse relationship between Lp(a) and glucose levels is observed only starting in prediabetes (Paige et al. 2017). The significance of this observation is not yet clear. There was also no association between isoform size and risk of diabetes (Kamstrup and Nordestgaard 2013). One hypothesized mechanism that explains this observation is that insulin suppresses apo(a) in hepatocytes. The biologic role of Lp(a) in insulin resistance and hyperglycemia requires more interrogation.

In those with cardiovascular disease, low levels of Lp(a) have also been associated with a greater prevalence of type II diabetes mellitus in prospective, retrospective, and genetic studies. In an analysis of 13,480 patients in the ODESSY OUTCOMES trial, similar findings were observed with negative correlation between Lp(a) and the prevalence of type 2 diabetes. Furthermore, in the same analysis, reduction of Lp(a) levels by the PCSK9 inhibitor alirocumab in those with high baseline Lp(a) level increased the estimated risk of incident type 2 diabetes compared with placebo hazard ratio 1.07 (95% CI 1.03–1.12; $P < 0.0002$) (Schwartz et al. 2021). However, there was an interaction between treatment with alirocumab and baseline Lp(a) on the risk of incident type 2 diabetes. The concentration of Lp(a) at which alirocumab had a neutral effect on incident type 2 diabetes was around 50 mg/dL. It was shown that PCSK9 inhibitor-induced reductions of Lp(a) levels lead to an increased risk of type 2 diabetes (Schwartz et al. 2021).

Although the mechanism by which low levels of Lp(a) contribute to an increased risk of type 2 diabetes is unknown, the risk may be modifiable as evidenced by Lp(a) levels in the above study that were neutral. Further, it seems that those with higher baseline Lp(a) who are treated with PCSK9 inhibitor therapy have an increased risk to develop type 2 diabetes than those with lower baseline Lp(a) levels (Schwartz et al. 2021). This observation, if confirmed in additional studies, is important to consider when prescribing this therapy to mitigate cardiovascular risk.

In summary, retrospective and prospective data have elucidated that Lp(a) levels are inversely related to an increased incidence of type 2 diabetes. The mechanism by which Lp(a) participates in diabetogenic pathophysiology is not yet understood. However, based on the studies discussed previously, the risk is modifiable through PCSK9 inhibitor therapy. Additional investigation is needed to understand whether Lp(a)-directed therapies currently in development impact risk for the development of diabetes.

Lp(a) in Wound Healing

Lp(a) is implicated in wound healing. Histologic studies have identified Lp(a) in all four stages of wound healing from infiltration of inflammatory cells to formation of granulation tissue. In the first stage of healing, there is an infiltration of inflammatory cells followed by formation of a fibrin clot mixed with red blood cells covers the exposed wound surface. In the second stage, the immature cell mass is replaced by granulation tissue, which is produced by fibroblasts, endothelial cells, and vascular sprouts from adjacent viable tissues, induced by growth factors released during the first stage. In this stage, granulation tissue is often covered with loose fibrous connective tissue with various thickness, which forms the fibrous cap. Angiogenesis also takes place in this stage. The epithelial sheets are spread to cover the granulation tissue in the third stage. In the last stage, collagen fibers replace the granulation tissue, resulting in reduction of wound size. Finally, the healing process is completed by replacement of granulation tissue with new epithelium or by organization (Yano et al. 1997).

The apo(a) and apoB100 subunits of Lp(a) are more strongly identified in the fibrous cap, endothelial cells, and plasminogen and fibrinogen-rich surfaces than in the re-epithelized tissue surface. The mechanism by which Lp(a) influences wound healing has not been described. Following from discussions elsewhere of Lp(a)'s role in angiogenesis and antifibrinolysis, it is possible that Lp(a) promotes the proliferation of endothelium with accompanying vasculogenesis; and is also involved in maintenance of the fibrin cap and preventing excessive fibrinolysis (Yano et al. 1997).

Lp(a) in Autoimmune Disease

Lp(a) elevation is associated with several autoimmune diseases (Missala et al. 2012; Toms et al. 2011). The mechanisms by which Lp(a) contributes to autoimmune disease are through acute phase reactions, autoantibodies, and fibrinolysis (Missala et al. 2012). The interplay of these mechanisms leads to increased inflammation which contributes to clinical autoimmune phenotypes.

Elevated Lp(a) is associated with increased circulating levels of acute phase reactants. In patients with rheumatoid arthritis, Lp(a) levels are associated with elevated C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) (Asanuma et al. 1999). Based on this association, Lp(a) is thought to have a crucial role in the acute inflammation pathway (see discussion of Lp(a) in inflammation previously). Observations of Lp(a) as an acute phase reactant have been identified in other inflammatory conditions such as polymyalgia rheumatica (Missala et al. 2012).

Autoantibodies are a prototypical mechanism of autoimmune disease. Autoantibodies to Lp(a) have been detected in antiphospholipid syndrome (APLS) and lupus. Specifically, for APLS, malondialdehyde (MDA)-modified lipoprotein(a) antibodies were observed. Antibodies reacting against MDS implicate increased oxidation in the pathogenesis of this condition (Romero et al. 1999).

Many autoimmune diseases carry an increased risk for cardiovascular disease, namely atherosclerotic plaque formation (Missala et al. 2012). For example, in those with rheumatoid arthritis, mortality from CVD is about 50% greater than controls. The increased CVD risk conferred by autoimmune diseases is attributed to dyslipidemia, systemic inflammation, and increased typical CVD risk factors, such as hypertension and obesity. Lp(a) seems to be at the crossroads between autoimmune disease and atherosclerosis. As discussed elsewhere, oxidized and glycosylated Lp(a) contributes to atherosclerotic plaque formation. In patients with rheumatoid arthritis and APLS, a similar glycosylated Lp(a) product is observed in the serum, specifically beta(2) GP1-Lp(a). Beta(2) GP1-Lp(a) is known to be associated with CAD and acute coronary syndromes. This product likely contributes to increased atherosclerotic risk in these populations in addition to typical CVD risk factors. There is limited data regarding the impact of Lp(a) on CVD in lupus. However, some observational studies have shown that Lp(a) is more elevated in those with CVD and lupus than those with lupus alone. Further research is needed to elucidate the mechanism of how Lp(a) promotes atherosclerosis in the setting of autoimmune disease (Wang and Zhang 2019; Missala et al. 2012).

Given its implication in the mechanism of autoimmune diseases, Lp(a) may be a viable target of intervention. Lp(a) lowering therapy may help reduce the systemic inflammation seen in these diseases and simultaneously mitigate the CVD risk. This will have to be evaluated prospectively in randomized trials with agents that specifically reduce Lp(a).

Lp(a) in Calcific Aortic Stenosis

Calcific aortic valve stenosis (CAVS) is the most common valve disease in the elderly population, affecting >1 million patients in the USA, and is associated with significant morbidity and mortality (Guddeti et al. 2020). Elevated Lp(a) is linked to increased risk for calcific aortic valve stenosis (CAVS). Observational studies

from the early 1990s showed that increased Lp(a) levels were associated with aortic valve calcification and stenosis. This relationship is linear, with higher Lp(a) levels correlating with higher risk. Multivariate analyses have shown that increased Lp(a) is an independent predictor of developing CAVS. Additionally, both prospective and retrospective genetic studies have shown that the LPA locus carries a greater risk of CAVS and may be causative. Interestingly, some studies have shown aortic stenosis after the sixth decade does not correlate with Lp(a) levels (Guddeti et al. 2020).

The relationship between Lp(a) and CAVS is driven in part by oxidized phospholipids. Oxidized phospholipid apoB-100 was linked to faster progression of aortic stenosis in those with elevated Lp(a). This observation leads to the postulation that Lp(a) leads to aortic valve stenosis by phospholipid oxidation. Mechanistically, OxPL are proinflammatory, can lead to endothelial dysfunction, and promote osteogenic differentiation which leads to calcification. Interestingly, the relationship between aortic stenosis progression and OxPL content may be linear, based on a subgroup analysis of the ASTRONOMER (Effects of Rosuvastatin on Aortic Stenosis Progression) clinical trial (Vavuranakis et al. 2020).

Increased Lp(a) levels have also been associated with valve calcification in patients with bicuspid aortic valves. Fewer KIV-2 repeats have also been linked to more severe calcification. In the context of calcification of a bicuspid aortic valve, Lp(a) could be a useful marker to identify those at risk to develop valve calcification and stenosis (Guddeti et al. 2020).

CAVS is often present in the setting of CAD. Interestingly, the relationship between Lp(a) and CAVS is independent of CAD. However, studying patients with this comorbidity has yielded novel insights into the mechanism of how Lp(a) promotes CAVS. Autotaxin (ATX), a lysophospholipase D enzyme, transforms lysophosphatidylcholine into lysophosphatidic acid (LysoPA). ATX is transported in the aortic valve via the bloodstream by Lp(a) and is also secreted by valve interstitial cells. ATX-LysoPA has been shown to promote inflammation and leads to calcification of the aortic valve, thus promoting CAVS. Autotaxin also indirectly promotes the nuclear translocation of the transcription factor NF-κB, which leads to heightened inflammation (Nsaibia et al. 2016).

In addition to deposition of oxidized phospholipids and inflammation mediated by autotaxin, Lp(a) has other pleiotropic mechanisms that lead to CAVS. Lp(a) is thought to participate in cholesterol deposition on the aortic valve cusps causing thickening. Lp(a) is also implicated in macrophage apoptosis and might contribute to early valve lesion progression. Further, following from Lp(a)'s role in thrombosis, it can cause fibrin deposition on the leaflets which can cause stenosis (Guddeti et al. 2020; Vavuranakis et al. 2020; Nsaibia et al. 2016).

In summary, Lp(a) contributes to the pathogenesis and progression of calcific aortic stenosis. Lp(a) has several pleiotropic mechanisms that contribute to CAVS, including, cholesterol deposition, delivery of OxPL, fibrin deposition, and inflammation mediated by autotaxin. More research is needed to determine if Lp(a) lowering therapy can mitigate the development of calcific aortic stenosis.

Lp(a) in Acute Coronary Syndromes

Elevated Lp(a) leads to an increased risk of coronary artery disease (CAD) and cardiovascular events (Tsimikas et al. 2020). Lp(a) contributes to atherosclerosis independent of LDL-C by many of the mechanisms discussed previously. These mechanisms include delivery of oxidized phospholipids and promotion of inflammation and thrombosis (Rehberger Likozar et al. 2020). Additionally, Lp(a) is associated with IL-8, a proinflammatory, prothrombotic, and proatherogenic cytokine, which attracts leukocytes, triggers tissue factor production, and promotes adhesion of monocytes to early atherosclerotic plaques (Lippi et al. 2021).

Observational data of the role of Lp(a) in CAD have shown that Lp(a) levels are higher in the setting of stable angina compared to unstable angina. Immunohistochemically, 90% of the Lp(a) area in coronary atheromas co-localizes with plaque macrophages, and 30% of which correlates with plaque a-actin, which might be related to the role of Lp(a) in plaque enlargement (Dangas et al. 1998). Similarly, in acute myocardial infarction (MI), Lp(a) levels increase significantly within the first 24 h and normalize within about 30 days (Rehberger Likozar et al. 2020).

The compete mechanism of how Lp(a) contributes to atherogenesis is not yet fully understood. In addition to the mechanisms discussed previously, it is proposed that Lp(a) is deposited on the vascular wall and is readily taken up by macrophage scavenger receptors. The macrophages soon become foam cells and the canonical pathway of atherogenesis follows. Lp(a) also induces endothelial dysfunction which is proatherogenic (van der Valk et al. 2016; Rehberger Likozar et al. 2020). As detailed previously, Lp(a) leads to coronary thrombi formation by antifibrinolysis (i.e., competitive inhibition of tPA). In addition, Lp(a) promotes coagulation and platelet aggregation and boosts inflammation (Boffa and Koschinsky 2016).

Based on the increased CVD mortality conferred by increased Lp(a) levels, current European Society of Cardiology guidelines recommend screening at least once. The 2018 American College of Cardiology/American Heart Association guidelines on blood cholesterol defined Lp(a) 50 mg/dL, or 125 nmol/L, as a risk-enhancing factor; according to their guidelines, this is a relative indication for its measurement with a family history of premature CVD. Those with elevated Lp(a) >180 mg/dL carry a risk of atherosclerotic CVD equivalent to patients heterozygous for familial hypercholesteremia (Rehberger Likozar et al. 2020).

Multiple studies have shown that the association of genetically predicted Lp(a) levels with the risk of CVD is independent of changes in LDL cholesterol levels. This is thought due to genetic variants that mimic the LDL lowering effects of statins, PCSK9 inhibitors, and ezetimibe to the risk of CVD (Burgess et al. 2018). This observation is more significant in younger patients. In those less than 45 years old, in whom elevated Lp(a) levels (>120 nmol/L, 80th percentile) are associated with a threefold increased risk of MI. The clinical benefit of lowering Lp(a) levels is proportional to the absolute reduction in Lp(a) levels. An absolute reduction in Lp(a) levels of approximately 100 mg/dL should result in a clinically relevant

reduction in the risk of CVD. Such a decrease in Lp(a) represents the same magnitude of CVD risk reduction achieved by lowering LDL cholesterol levels by 38.67 mg/dL (Tsimikas et al. 2020; Rehberger Likozar et al. 2020).

Specifically, in acute coronary syndromes (ACS), Lp(a) elevation is observed for up to 4 months after an event. Concomitantly, OxPL levels are also elevated, which could mean that Lp(a) participates in OxPL delivery during acute plaque rupture. Interestingly, Lp(a) and OxPL transient elevations have also been observed after percutaneous coronary intervention for stable CAD (Tsimikas et al. 2020). Lp(a) levels are also inversely related to the age of first presentation with ACS. This means that younger patients presenting with ACS or observed to have higher Lp(a) levels than older patients with a similar ACS presentation. This reflects the importance of other, traditional atherosclerotic risk factors in older individuals, in contrast to a more important role of Lp(a) in younger individuals (Vavuranakis et al. 2020).

Evolution of Lp(a)

The synthesis of apo(a) is confined to a certain group of primates. However, the hedgehog produces an apo(a)-like protein composed of tandem repeats of plasminogen kringle III homologous domain but without the protease domain (Lawn et al. 1997). Phylogenetic analysis has determined that the human and hedgehog genes evolved independently from different DNA sequences. This observation signifies convergent evolution (Lippi and Guidi 2000).

The human apo(a) is in a 400 kb gene cluster on the telomere of chromosome 6 (6q26-27) (Frank et al. 1988). Sequencing of the apo(a) gene has revealed it contains ten different kringle IV subtypes. There are at least 34 different polymorphisms within the plasminogen kringle IV type 2 domain. This contributes to allelic heterogeneity among the many apo(a) isoforms identified in human plasma (Lippi and Guidi 2000).

Interestingly, there are also null alleles of the apo(a) gene that produce no circulating Lp(a) (Cox et al. 1998). This is caused by an in frame 47 amino acid deletions in the protease domain. This precludes the correct splicing of apo(a) mRNA, which creates a nonfunctioning protein. This is thought to lead to improper post-translational N terminal glycosylation (Lippi and Guidi 2000). The apo(a) gene cluster also includes the sequences encoding plasminogen. Apo(a) is homologous to three other genes, which are together termed plasminogen-related genes. These are located on chromosome 2 and 4 (Lippi and Guidi 2000).

Apo(a) has sequence homology with a diverse gene family (Byrne et al. 1994; Magnaghi et al. 1994). This includes genes that encode proteins that are involved in thrombosis and coagulation, such as, prothrombin, tissue type plasminogen activator (t-PA), and factor XII. Apo(a) also shares sequence homology with macrophage-stimulating factor and hepatocyte growth factor, the sequence homology of apo(a) to these other genes informs on the structure and function relationship. This means that the genes that apo(a) shares homology with inform on its mechanism (Lippi and Guidi 2000).

Further genetic analysis has elucidated the boundaries of intron and exon sequences are remarkably similar between these genes and only differ between 1% and 5% (Lawn et al. 1997; Ichinose 1992). Also, the intron and exon junction positions are almost identical. These findings suggest that apo(a) and these other genes may have developed during recent primate evolution from a common ancestral component of the kringle-related serine protease, most likely plasminogen, via duplication and exon shuffling (Lippi and Guidi 2000).

The apo(a) gene is most homologous with the proenzyme plasminogen. They both share the Kringle V domain. However, the kringle IV domain of plasminogen is in the apo(a) gene as multiple variable tandem repeats (McLean et al. 1987). Furthermore, a point mutation in the domain homologous to the protease domain of plasminogen deprives apo(a) of enzymatic activity (Lippi and Guidi 2000).

It is evident that Lp(a) has been conserved through evolution. This may be due to its positive influence on wound healing, thrombosis in the face of injury and, when at high concentrations, may be protective against diabetes. Its structural similarities to other local genes inform on its diverse functions. Lp(a) has a complex mechanism of action and although may seem deleterious in some contexts; it could also confer an evolutionary advantage that is not yet fully understood.

Lp(a) and COVID-19

COVID-19 infection has led to increased mortality in those with significant pulmonary disease, ischemic heart disease, diabetes, and human immunodeficiency virus infection (Enkhmaa and Berglund 2022). COVID-19 infection leads to a hyperinflammatory state that is linked with an increased risk of venous thromboembolism and cardiovascular complications (Enkhmaa and Berglund 2022).

It was hypothesized that Lp(a) has a synergistic effect with COVID-19 infection. This follows from its proinflammatory and prothrombotic roles discussed elsewhere. Briefly, because Lp(A) is an acute phase reactant and contributes to inflammation, in part, by carrying oxidized phospholipids. Regarding thrombosis, one major mechanism is the inhibition of endogenous fibrinolysis (Boffa and Koschinsky 2016). Furthermore, the stimulation of IL-6 from COVID-19 infection was thought to promote the acute phase expression of Lp(a). When the relationship between Lp(a) levels and COVID-19 infection was interrogated, there was no significant difference in the serum Lp(a) levels between those infected with COVID-19 and controls (Enkhmaa and Berglund 2022).

However, Lp(a) levels increase over the course of hospitalization and increase in concentrations that are associated with the severity of COVID-19 infection and stage of acute kidney injury in these patients. In the COVID-19 population in a small observational study, Lp(a) was not associated with IL-6, a well-known inflammatory marker. Taken together, these results suggest that a hyper Lp(a) state,

independent of inflammation, may lead to severe COVID infection with kidney injury (Lippi et al. 2021; Nurmohamed et al. 2022). Lp(a) was positively associated with IL-8, which is a cytokine involved in coronary atherosclerosis, venous thromboembolism, and thrombotic microangiopathy. Further, Lp(a) was negatively associated with ADAMTS13 (a von Willebrand factor-cleaving protease) and von Willebrand factor. These observations suggest a thrombotic microangiopathy in the pathogenesis of severe COVID-associated acute kidney injury (Lippi et al. 2021).

The absence of an association between Lp(a) and COVID-19 infection is contrary to several other studies that have observed elevated Lp(a) levels in inflammatory diseases (Toms et al. 2011; Romero et al. 1999). One explanation as to why this was not observed in COVID-19 infection is that Lp(a) elevation may occur after chronic exposure. In addition, relative increases were observed in patients while hospitalized (Enkhmaa and Berglund 2022).

Despite Lp(a) levels not being significantly more elevated in those with COVID-19 infection, the SARS-CoV-2 infection enhanced the associations of elevated Lp(a) concentrations with atherosclerotic events such as ischemic heart disease. The mechanism of this may be due to vascular preconditioning from chronic Lp(a) exposure which may make the endothelium more susceptible to COVID-19-induced inflammation. In addition, Lp(a) is associated with IL-8 in COVID-19 infection. As discussed elsewhere, IL-8 promotes atherosclerotic plaque instability. More research is needed to fully elucidate this mechanism. Lastly, how gender and sex differences influence the relationship between Lp(a) and COVID-19 infection is not understood at this time.

There was no association observed between COVID-19 infection and increased risk for venous thromboembolism in those with elevated Lp(a) (Enkhmaa and Berglund 2022). However, it was observed in hospitalized COVID-19 patients who had the largest increase in Lp(a) relative to admission had an increased incidence of VTE. The discordance in these observations may be explained by the fact that Lp(a) by itself is not a prothrombotic factor; but rather an antifibrinolytic and thus predominantly may cause clot-propagation in pre-existing thrombi as a "second hit" agent. This "second hit" mechanism could already be activated at relatively low Lp(a) levels, even below the ASCVD risk threshold of 50 mg/dL from the 2019 ESC/EAS guidelines. In the case of COVID-19, severe endothelial injury and ongoing active coagulation may be particularly sensitive to Lp(a) tipping the balance to clot propagation and clinical expression of VTE (Nurmohamed et al. 2022).

In summary, Lp(a) is associated with severity of COVID-19 infection and development of severe AKI. Lp(a) serum concentrations have been observed to increase over the course of infection in hospitalized patients. Patients with the greatest increase observed to have a greater incidence of VTE. COVID-19 infection in those with elevated Lp(a) at baseline is associated with increased atherosclerotic disease. More research is needed to elucidate the mechanism of these observations and if Lp(a) lowering therapies have a role in the treatment of COVID-19.

Conclusions

1. Lp(a) is highly versatile and participates in a broad range of physiological phenomena that can have both good and bad biochemical and histological consequences.
2. It will require a great deal of additional investigation to further delineate how specific kringle domains, phospholipids, and the Lp(a) particle as a whole is capable of driving such a wide spectrum of biochemical phenomena. Its proteome and lipidome require much additional characterization. It will also be of interest to determine if this lipoprotein, like others, can carry micro RNAs and if its biochemical cargo varies as a function of the physiological milieu.
3. Work is ongoing with prospective randomized clinical trials using pharmacologic interventions to lower Lp(a). It will clearly be of interest to determine if Lp(a) lowering results in a reduction of risk for acute cardiovascular events.
4. Whether Lp(a) lowering can be harnessed to reduce aortic valve calcification, risk for thromboembolic phenomena, some forms of malignancy, and attenuate inflammation will also be of interest.
5. It will also be important to establish whether or not therapeutic Lp(a) lowering associates with increased risk for diabetes mellitus, impaired wound healing, and possible toxicity from reduced oxidized phospholipid transport.

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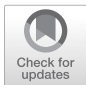
Chapter 9
The Role of Lp(a) in Atherosclerosis: An Overview



Anastasiya Matveyenko, Marianna Pavlyha, and Gissette Reyes-Soffer

Introduction

Lipoprotein(a) [Lp(a)] is an apolipoprotein B100 (apoB100)-containing particle that circulates in human plasma. It differs from other apoB lipoproteins by its lipid composition and the presence of a covalently bound glycoprotein, apolipoprotein(a) [apo(a)] (Berg 1963; Jawi et al. 2020). Recently published studies in cell models have also described noncovalent bonding between apo(a) and apoB (Youssef et al. 2022). Plasma levels of Lp(a) are genetically regulated and variations can be traced to the *LPA* gene locus (Utermann 1989). The gene has large homology with the plasminogen gene. Similarities between them and effects on pathophysiology are still being investigated (Zheng et al. 2020). Unlike other apoB-containing lipoproteins, the apo(a) within Lp(a) has a broad range of sizes from 300 to 800 kDa (Lackner et al. 1991). This is due to the number of Kringle IV type 2 (KIV-2) repeats, resulting in apo(a) isoforms ranging from 1 to greater than 40 KIV-2 repeats (Kostner and Kostner 2017; Utermann 1999). Individuals can express one or two apo(a) isoforms, which are synthesized in the liver and then bind to apoB particles. There are particle composition similarities between Lp(a) and other apoB-containing lipoproteins, and even HDL (Scanu 1988). However, the mechanisms that regulate the synthesis and distribution of Lp(a) are not completely defined and can be independent from LDL- and HDL-described functions. Lp(a) synthesis takes place inside hepatocytes with likely association to apoB100 on the cell surface. There is

Anastasiya Matveyenko and Marianna Pavlyha contributed equally with all other contributors.

A. Matveyenko · M. Pavlyha · G. Reyes-Soffer (✉)
Division of Preventive Medicine and Nutrition, Department of Medicine, Vagelos College of Physicians and Surgeons, Columbia University, New York, NY, USA
e-mail: am4498@cumc.columbia.edu; mp3301@cumc.columbia.edu; gr2104@cumc.columbia.edu

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_9

no evidence that Lp(a) levels in plasma are related to lipoprotein lipase activity, and it is unlikely that it is derived from catabolism of other lipoproteins. Similarly, Lp(a) clearance may be regulated by various pathways (Reyes-Soffer and Ramakrishnan 2017; Chemello et al. 2022), depending on the particle composition. Several studies show that Lp(a) clearance can be dependent on LDL receptors; however, studies with PCSK9 inhibitors varied in their results (Reyes-Soffer and Ramakrishnan 2017; Chemello et al. 2022). The latter may be related to the use of statins and differences in ethnicities of the cohorts evaluated (Reyes-Soffer and Ramakrishnan 2017; Chemello et al. 2022). Large studies have shown an inverse relationship between Lp(a) plasma concentrations and isoform sizes, based on the KIV-2 repeats (Sandholzer et al. 1992; Kraft et al. 1992; Stefanutti et al. 2020). This relationship can account for 30–70% of the plasma levels. Notably, Black and Asian Indian ethnicities have higher levels of Lp(a) when compared to Caucasians, pointing again to genetics as being one of the determining factors (Kronenberg and Utermann 2013; Enkhmaa and Berglund 2016; Reyes-Soffer et al. 2021; Patel et al. 2021). Additionally, small differences in Lp(a) levels have been found between men and women (Markus et al. 2021; Forbang et al. 2016; Simony et al. 2022).

Lipoprotein(a) and Atherosclerotic Cardiovascular Disease

Elevated levels of Lp(a) are causal for atherosclerotic cardiovascular disease (ASCVD) (Reyes-Soffer et al. 2021), as confirmed by epidemiological (Nordestgaard et al. 2010; Bennet et al. 2008), Mendelian randomization (Burgess et al. 2018), and genome-wide association studies (GWAS) (Kettunen et al. 2016; Tybjaerg-Hansen 2016; Nordestgaard and Langsted 2016). A large epidemiological study, looking at records of over 6000 participants, found an association between Lp(a) levels and Coronary Heart Disease (CHD) risk that remained regardless of adjusting for other known risk factors such as diabetes, hypertension, lipids, and smoking status (Bennet et al. 2008). This suggests that unlike triglycerides and C-reactive protein, which are affected by these risk factors, the relationship between CHD and Lp(a) is not. A Mendelian randomization study analyzed data from 62,240 patients with CHD versus 127,299 controls and reported that the association of genetically predicted Lp(a) with CHD risk was linearly proportional to the absolute change in Lp(a) levels (Burgess et al. 2018). Another group performed an extended genome-wide association study with 24,925 individuals and found that genetic variation in *LPA* appears to be associated with ischemic heart disease and provides support for treatment of high Lp(a) levels for CHD risk reduction (Kettunen et al. 2016). ASCVD has a complex biology and pathophysiology leading to various clinical presentations. A therosclerosis starts with lipid build up in arterial wall, followed by inflammatory cascase activation, and cell turnover (Libby et al. 2019). The last stage of atherosclerotic progression - destabilization of lipid-rich plaque is what

ultimately leads to events, such as myocardial infarction, stroke, and associated increase in mortality (Libby 2013). The time course of disease development varies due to the numerous interlinks of metabolic risk factors.

The role of apoB-containing lipoproteins in this process has been well-established (Sniderman et al. 2019). Specifically, Lp(a) is known to have proatherosclerotic, prothrombotic, and proinflammatory roles (Tada et al. 2019; Riches and Porter 2012) (Fig. 9.1). Some key signatures of its atherosclerotic profile include (Berg 1963) endothelium injury, (Jawi et al. 2020) development of lipid deposition, i.e., fatty streak within the vessel intima compartment, (Youssef et al. 2022) presence of leukocytes and deposition of smooth muscle cells into the vascular wall, (Utermann 1989) presence of foam cell, i.e., macrophages, and (Zheng et al. 2020) degradation of the extracellular matrix (Kobiyama and Ley 2018). Excessive uptake of Lp(a) by

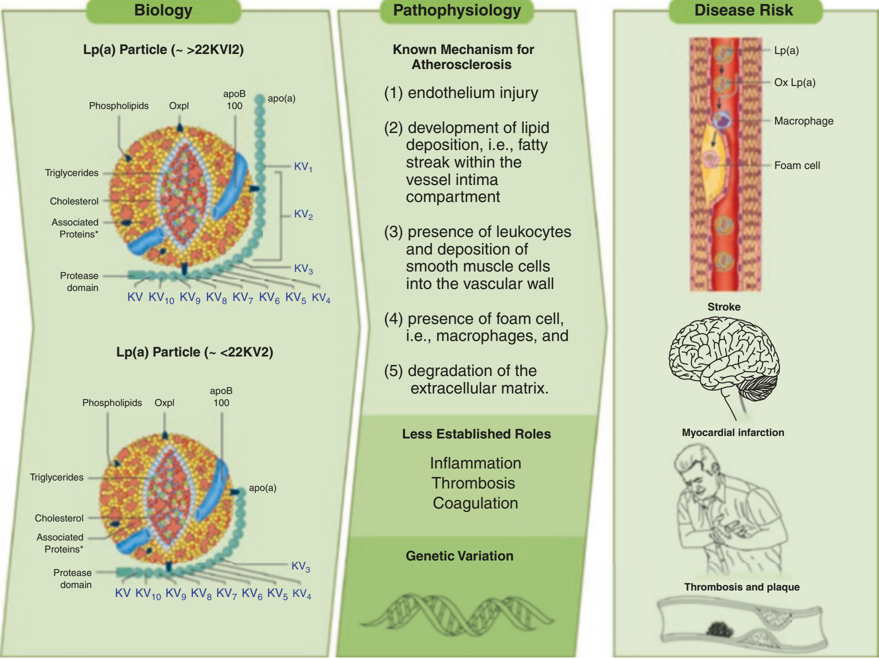

Fig. 9.1 Lipoprotein(a): biology, pathophysiology, and disease development. (Panel **a**) The structure and function of circulating lipoprotein particles have been nicely described. Proteins (*) on the lipoprotein(a) particle have led to further understanding of its link to disease development (McCormick and Schneider 2018). (Panel **b**) Lp(a) has been linked to atherosclerosis and the roles of genetics and the additional proteins bound to the particle are still to be fully described (Kronenberg 2022). (Panel **c**) As an apoB100-containing particle, Lp(a) can lead to plaque formation, yet, further research is needed to understand its link to specific disease presentation. Genome-wide association, epidemiological, and Mendelian randomization studies support its role as causal in development of ASCVD

macrophages with subsequent transformation into foam cells sheds light onto its integral role in atherogenesis. The role of Lp(a) in coronary artery disease (CAD) has been studied previously (Rasouli et al. 2006), with a recent study reaffirming that high Lp(a) levels are associated with increased progression of coronary low-attenuation plaque (necrotic core) between baseline and 12 months of follow-up in patients with advanced stable CAD (Kaiser et al. 2022). The role of Lp(a) in these specific atherogenic processes has been studied by various authors (Marchini et al. 2021), but its exact pathophysiology, not related to conventional apoB injury, has not been completely described.

Lipoprotein(a) and Links to Inflammation

Advancements in mass spectrometry have enhanced the ability to study Lp(a) composition further. These new tools have led to identification of novel pathways unique to Lp(a) which can explain the pathogenic nature of Lp(a) and its link to ASCVD (Rodger et al. 2018; McCormick and Schneider 2018). Proteomic studies of Lp(a) mouse models, which present elevated levels of both LDL and Lp(a), showed that the apo(a) protein allows Lp(a) particle to bind more oxidized phospholipid (OxPl) molecules than LDL, possibly involving antioxidant enzymes, glutathione peroxidase 1 and peroxiredoxin 6 (Rodger et al. 2018). The binding of OxPls to Lp(a) has been investigated as one of the possible culprits of its atherogenicity (Stefanutti et al. 2020). Upon binding, there is an initiation effect of Lp(a) on macrophages, leading to increased IL-8 expression. Notably, those individuals who have higher levels of Lp(a) may have a higher potential for oxidized phospholipid binding and subsequent atherogenic activation of Lp(a) (Berliner and Watson 2005).

There is also an association between Lp(a) and several inflammatory conditions. This is particularly interesting, as inflammation has been shown to be directly involved in ASCVD risk (Ridker et al. 2017). For example, a positive relationship between Lp(a) and interluekin-6 (IL-6) has been suggested in subjects with chronic inflammatory conditions. Additionally, some studies show that Lp(a) may serve as a chemoattractant for monocytes and affect IL-6 via this pathway as well (Syrovets et al. 1997). It also influences the expression of vascular cell adhesion molecule (VCAM)-1, E-selectin, and intracellular adhesion molecule (ICAM) in endothelial cells (Schnitzler et al. 2020). All these play an integral role in the early plaque development. Interestingly, during MI, monocyte levels increase dramatically resulting in an inflammatory response (Nahrendorf et al. 2010). Additionally, Lp(a) plays a major role in calcific aortic valve stenosis (CAVS). The latter highlighted by human proteomic studies that found lifelong exposure to elevated Lp(a) contributes to the development and progression of CAVS through multiple pathways (Bourgeois et al. 2021).

Lipoprotein(a) and Atherosclerosis: Brief Review of Clinical Outcomes

Myocardial Infarction

Multiple studies have demonstrated the association between Lp(a) and myocardial infarctions (MIs), although these were mostly conducted in Caucasian populations (Afanasieva et al. 2022). Lp(a) levels greater than 50 mg/dL were linked to greater risk of developing an MI (Kamstrup et al. 2008). A study looking at a Danish population showed that there was a step-wise effect with Lp(a) levels and risk of MI (Emerging Risk Factors Collaboration et al. 2009; Erqou et al. 2009; Langsted et al. 2015). Particularly those patients with Lp(a) levels in the 95th percentile had as high as three to fourfold risk for an experiencing at event. This risk was noted to be higher in men compared to women.

Aortic Stenosis

There is a strong association between Lp(a) and Aortic Stenosis (AS) with main driver in the mechanism of the disease being oxidized phospholipids. There is also an association between higher Lp(a) measurements with a more rapid progression of stenosis and greater need for aortic valve replacement compared to the group with lower Lp(a). Exposure of valvular interstitial cells to Lp(a) increases the expression of the osteoblastic transcription factors, runt-related transcription factor 2 (RUNX2) and bone morphogenetic protein 2 (BMP2), suggesting that Lp(a) plays a role in osteogenic differentiation of valvular interstitial cells with oxidizing phospholipids playing an integral role in this mechanism (Zhiduleva et al. 2018). Additionally, Lp(a) plays a major role in calcific aortic valve stenosis (CAVS) Calcific aortic valve stenosis (CAVS). The latter highlighted by human proteomic studies that found lifelong exposure to elevated Lp(a) contributes to the development and progression of CAVS Calcific aortic valve stenosis (CAVS) through multiple pathways (Bourgeois et al. 2021).

Stroke

There has been conflicting data regarding the association between levels of Lp(a) and stroke (Colantonio et al. 2022; Pan et al. 2022). It is well established that Black populations are at a higher risk for CAD stroke, and mortality compared to other ethnicities and tend to have higher levels of Lp(a). The REasons for Geographic and Racial Differences in Stroke (REGARDS) study published data on Lp(a) and

stroke in a race and sex stratified cohort. Their findings, after adjusting for other risk factors, showed a correlation between high Lp(a) levels and ischemic stroke with higher hazard ration in Black populations. Women had higher on average Lp(a) levels, but no statistically significant association with stroke. The driving mechanisms are thought to be similar as in CAD and PAD, involving increased cholesterol deposition in plaque, inflammation, and prothrombotic effects (Arora et al. 2019).

Peripheral Artery Disease (PAD)

While there are numerous publications linking Lp(a) and CAD data pertaining to PAD are not as robust (Norgren et al. 2007). Review of current publications does, however, suggest that elevated levels of Lp(a) are associated with increased incidence, progression, and post-treatment recurrence of PAD (Tmoyan et al. 2018). The pathophysiology behind this relationship is driven by the ability of Lp(a) particles to migrate more easily into the subendothelial space when compared to LDL particles (Kraaijenhof et al. 2021). This is facilitated by endothelial activation via oxidized phospholipids and upregulation of chemokines and adhesion molecules. Additionally, Lp(a) has been shown to compete for binding of plasminogen and plasmin, generating a prothrombotic state (Boffa 2022). The latter is especially important for those patients with limb threat due to tibial disease (below the knee) (Tsimikas 2017). High Lp(a) levels have been shown to be associated with increased incidence of claudication, symptom progression, re-stenosis after intervention, hospitalization due to PAD, and limb amputation (Price et al. 2001). Patients with elevated Lp(a) also have higher risk of combined PAD outcomes after adjusting for other traditional risk factors (Kosmas et al. 2019). A recent prospective, observational study in symptomatic lower extremity arterial disease comparing patients with high and low Lp(a) was performed. It showed that compared with low-Lp(a) group, patients with high-Lp(a) had a higher proportion of heart failure, CLTI, and multivessel lesions as well as higher LDL cholesterol. A 5-year incidence of all-cause mortality was significantly higher in the high Lp(a) cohort than in those with low Lp(a) (48.1% vs. 27.3%). Additionally, the cumulative 5-year incidence of major adverse limb occurrence was also significantly higher in patients with high Lp(a) levels (67.9% vs. 27.2%) (Tomoi et al. 2022).

Lp(a): Cardiovascular Mortality

Lp(a) is a known independent risk factor for increased mortality. A study published in 2019, examining a Danish population, reported high risk of both cardiovascular and all-cause mortality with no difference in noncardiovascular-related mortality (Langsted et al. 2019). Reported median survival was the lowest in those patients

who had the highest measured Lp(a) (>93 mg/dL). The known causal factors driving high mortality in patients with Lp(a) are mainly coronary heart disease, myocardial infarction, atherosclerotic stenosis, and aortic valve stenosis (Nordestgaard and Langsted 2016). Another cross-sectional study done in United Kingdom looking at a large cohort of patients determined via Mendelian randomization that genetically elevated Lp(a) levels were associated with parental life span. High Lp(a) levels were also shown to be associated with increased all-cause mortality (Patel et al. 2021; Arsenault et al. 2020).

Lipoprotein(a): Genetics and Atherosclerosis

Multiple genetic studies and significant observations of a link between Lp(a) and cardiovascular disease risk have been published (Mehta et al. 2020; Arsenault and Kamstrup 2022). These focus on examining associations between Lp(a) and cardiovascular risk, CHD, lifespan, using genetic make-up, including family history, to better understand genetic role of Lp(a) in ASCVD. The role of genetic variants outside and within the KIV2 region of the *LPA* gene have been recently described. There have been numerous variants proposed for CAD (Clarke et al. 2009). In studies by Clarke et al., a number of chromosomal regions were associated with risk of CAD, and the *LPA* locus region 6q26–27 has the strongest relationship between high Lp(a) levels and risk of CAD (Clarke et al. 2009). Various single nucleotide polymorphisms (SNPs), rs10455872 and rs3798220 within the *LPA* site, have been described and are highly associated with high Lp(a) levels. These variants are more common in those of European ancestry. In the work of Kamstrup and Nordestgaard, the genotypes mentioned above and high Lp(a) levels were associated with an increased risk of heart failure, consistent with causal association (Kamstrup and Nordestgaard 2016). More recently, the same authors have highlighted the effects of Lp(a) on morbidity and mortality (Simony et al. 2022). Beyond the effect of specific single nucleotide polymorphisms (SNPs), possible SNP-SNP interactions and SNPs in the KIV-2 repeat region have to be taken into account, which might not be picked up by conventional sequencing methods (Coassin and Kronenberg 2022). Work in the cohort from Pakistan (Saleheen et al. 2017) at risk for myocardial infarction showed additional SNPs and that both, smaller apo(a) isoform size and high Lp(a) levels, are independent and causal risk factors for CAD. In studies of diverse cohorts, such as the Atherosclerosis Risk in Communities (ARIC), Lp(a) measured at middle age of participants was significantly associated with valvular and vascular calcification at older age, represented by aortic valve calcium, mitral valve calcification, and other factors (Obisesan et al. 2022). In this cohort, plasma Lp(a) levels and family history of cardiovascular disease had independent and additive joint associations with cardiovascular risk (Mehta et al. 2020). Another study, looking at mostly males from Southeast Asia, found that Lp(a) levels in plasma are a positive predictor of coronary artery disease and acute myocardial infarction (Loh et al. 2022).

Effects of Available Treatments on Lp(a) and Risk of Atherosclerosis

There are currently no targeted approved pharmacologic therapies that lower Lp(a) concentrations. However, some therapies lower apoB and LDL cholesterol (LDL-C), decrease Lp(a) modestly (niacin—20%; CETP inhibitor—24–36%; ApoB antisense therapy—26–27%; microsomal triglyceride transfer protein inhibitor—17%; Anti-IL6R—30–37%) (van Capelleveen et al. 2016). Proprotein convertase subtilisin:kexin type 9 (PCSK9) inhibition showed a 25% reduction in Lp(a), and after one year of treatment, reduced the event rate for acute cardiovascular events (Kaiser et al. 2022). However, that could be due to the combined effect of lowering LDL-C with Lp(a). Lipoprotein apheresis is approved for treatment of high Lp(a) for specific patients at increased risk in the United States (Nugent et al. 2020). Regular lipoprotein apheresis has been approved in Germany for lipoprotein(a) hyperlipoproteinemia with progressive cardiovascular disease since 2008 (Roeseler et al. 2016). A study conducted in Germany looked at 36,745 lipoprotein apheresis treatments of 118 patients to analyze the efficacy, safety, and tolerability (Heigl et al. 2015). Average annual rate of major adverse coronary events was reduced by 79.7% for all patients after beginning lipoprotein apheresis (Heigl et al. 2015). Overall, the procedure was well-tolerated and effective for CVD risk reduction (Heigl et al. 2015). There have been since developed apheresis preferential for Lp(a) using antibodies against apo(a), targeting people with high Lp(a) and otherwise normal lipid levels (Waldmann and Parhofer 2019). Likewise in the US, similar results were seen with the use of lipoprotein apheresis in patients with high Lp(a) and relatively normal LDL_C, showing an improvement in cardiovascular events (Moriarty et al. 2019). Various targeted treatment programs are in phase 2 and phase 3 of development (Reyes-Soffer et al. 2021), mostly decreasing the synthesis of apo(a) using biologicals and RNA inteferring agents (Tokgözoğlu and Libby 2022).

It is not clear whether decreasing apo(a) alone versus decreasing apoB and apo(a) will be beneficial (i.e., decrease event rate and mortality) to individuals with isolated high Lp(a) levels with these therapies. Furthermore, due to additional protein components associated with Lp(a) and links to disease mechanism (i.e., inflammation), other targets for treatments may be considered. These promising therapies may bring an additional benefit to those patients who are already at a high risk for CVD due to their elevated apoB and LDL-C and are on optimal therapy. 2019 European guidelines make recommendations to access Lp(a) level along with history of heart disease and other known risk factors when devising a long-term patient care plan for best future outcomes (Mach et al. 2019). Further randomized trials are needed to gain more insight whether these therapies or new treatments targeting Lp(a) will change patient management and disease progression.

Conclusions

Lp(a) is an atherogenic lipoprotein present in human plasma with higher levels corresponding to an elevated risks for ASCVD. It has a strong genetic predisposition and its mechanisms of action have been linked to the propagation of atherogenic cascade via alteration of macrophage gene expression. Although there are currently no widely prescribed Lp(a)-lowering treatments in the United States, there are available therapies, whose utility in clinical practice, needs to be further studied.

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Chapter 10
Molecular Mechanisms of Lipoprotein(a) Pathogenicity: Tantalizing Clues and Unanswered Questions

Michael B. Boffa and Marlys L. Koschinsky

Introduction

Although lipoprotein(a) (Lp(a)) was discovered almost 50 years ago (Berg and New 1963) and has been subsequently shown to be a causal and independent risk factor for atherosclerotic cardiovascular disease (ASCVD) and calcific aortic valve disease (CAVD) (Arsenault and Kamstrup 2022), the mechanisms by which Lp(a) mediates its pathogenic effects in vivo remain unclear. Lp(a) comprises an apoB-100-containing lipoprotein to which is attached the unique apolipoprotein(a) (apo(a)) moiety (Fig. 10.1). Amino acid analysis followed by complete sequencing of the human apo(a) cDNA in 1987 revealed a high level of sequence identity with the profibrinolytic enzyme plasminogen (Eaton et al. 1987; McLean et al. 1987). Apo(a) contains a series of tri-looped structures called kringles that are similar to the KIV domain of plasminogen, followed by sequences similar to the plasminogen KV and protease domains (Fig. 10.1). Due to several critical amino acid substitutions and a small deletion, the apo(a) protease-like domain has been shown to be catalytically inactive (Gabel and Koschinsky 1995).

M. B. Boffa (✉)
Robarts Research Institute, Schulich School of Medicine and Dentistry, The University of Western Ontario, London, ON, Canada

Department of Biochemistry, Schulich School of Medicine and Dentistry, The University of Western Ontario, London, ON, Canada
e-mail: mboffa@uwo.ca

M. L. Koschinsky
Robarts Research Institute, Schulich School of Medicine and Dentistry, The University of Western Ontario, London, ON, Canada

Physiology and Pharmacology, Schulich School of Medicine and Dentistry, The University of Western Ontario, London, ON, Canada
e-mail: mlk@robarts.ca

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_10

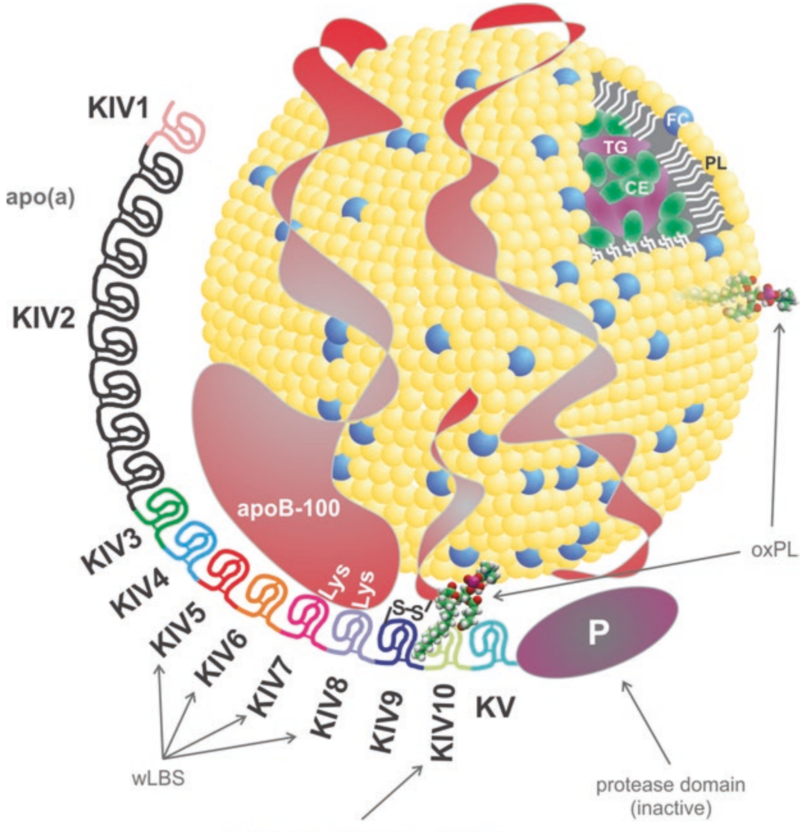

Fig. 10.1 Structure and functional domains of Lp(a). Lp(a) consists of apo(a) covalently linked to the apoB-100 moiety of an LDL-like lipoprotein particle. The lipid portion of the particle is a shell of phospholipids (PL) and free cholesterol (FC) surrounding a neutral lipid core of cholesteryl esters (CE) and triacylglycerols (TG). Apo(a) consists of ten types of KIV domains, a KV domain, and an inactive protease domain. KIV2 is repeated different numbers of times in different apo(a) isoforms. KIV5–KIV8 contain weak lysine-binding sites (wLBS), with those in KIV7 and KIV8 binding to specific lysine residues in apoB-1000 during the noncovalent step of Lp(a) assembly. KIV9 contains a single-free cysteine that mediates disulfide bond formation with apoB-100. KIV10 contains a strong lysine-binding site (sLBS) as well as covalently bound oxidized phospholipid (OxPL). The sLBS is required for OxPL addition, and together these features promote several pathogenic effects on vascular and immune cells. OxPL is also present noncovalently associated with the lipid moiety of Lp(a), and accounts for up to 50% of the total OxPL on Lp(a). *EC* endothelial cell, *SMC* smooth muscle cell, *VIC* valve interstitial cell

Apo(a) kringle IV sequences are present in ten types based on amino acid sequence; these have been designated KIV1–KIV10 (McLean et al. 1987; van der Hoek et al. 1993). The KIV2 sequence is present in a variable number of identically repeated copies (from 3 to greater than 40) which is a hallmark of Lp(a) and reflects allele size variation in *LPA*, the gene encoding apo(a) (Fig. 10.1) (Lackner et al. 1993; Marcovina et al. 1996). Interestingly, there is a strong inverse correlation between apo(a) size and Lp(a) plasma levels, which likely arises due to less efficient secretion of larger isoforms as a result of presecretory degradation of misfolded species in the endoplasmic reticulum (Boffa and Koschinsky 2022). The KIV9 domain houses the only unpaired cysteine in apo(a) and is involved in disulfide bond formation with a cysteine residue in the carboxyl-terminus of apoB-100 (Koschinsky et al. 1993). The KIV5–8 domains each contain a weak lysine-binding site (wLBS); the wLBS in KIV7 and KIV8 is required for intracellular noncovalent interaction between apo(a) and apoB that precedes extracellular disulfide bond formation (Fig. 10.1) (Becker et al. 2004; Youssef et al. 2022).

The apo(a) KIV10 domain contains a relatively strong lysine-binding site (sLBS) that has been studied extensively in attempts to understand the pathophysiology of Lp(a) in the vasculature. Lp(a) has been demonstrated to be the preferential lipoprotein carrier of proinflammatory oxidized phospholipids (OxPL), compared to LDL (Bergmark et al. 2008). These species are present both on the lipid portion of Lp(a) as well as covalently associated with apo(a) (Bergmark et al. 2008; Leibundgut et al. 2013). Interestingly, in this regard, it has been shown that the KIV10 sLBS is absolutely required for the covalent addition of oxidized phospholipid to this kringle, likely involving addition of the OxPL adduct to a histidine side chain through Michael reaction addition (Fig. 10.1) (Leibundgut et al. 2013; Scipione et al. 2015). The proinflammatory effect of the OxPL on KIV10 has been demonstrated in many studies, both in vitro and in vivo (Koschinsky and Boffa 2022; Dzobo et al. 2022). In vitro studies have shown the role of OxPL on KIV10 in promoting proinflammatory and phenotypes in a variety of vascular and inflammatory cells including valve interstitial cells (VICs) (see below).

Many studies using a variety of vascular cell types have shown that the apo(a) sLBS can compete with plasminogen for binding to cell surfaces, thereby inhibiting plasminogen activation to the active enzyme plasmin (Boffa 2022). Downstream effects on fibrin clot lysis have also been studied, with variable results, and the significance of Lp(a) in promoting thrombosis in the arterial and venous circulation remains controversial (Boffa 2022). Indeed, Lp(a) appears to confer risk for venous thromboembolism only in individuals with extremely high Lp(a) levels (Kamstrup et al. 2012). The role of Lp(a) in platelet function and coagulation, and in the lysis of platelet-rich clots, is not clear (Boffa 2022). Importantly, it is difficult to assess the contribution of Lp(a) to lysis of clots formed upon rupture of vulnerable atherosclerotic plaques (see below).

Despite the demonstration of elevated plasma Lp(a) levels as an independent and causal risk factor for ASCVD and CAVD, the mechanism of action of Lp(a) in these disease processes remains unclear. This reflects, in part, the complexity of the Lp(a) structure, as well as the lack of suitable animal models for Lp(a); together these

present significant challenges to understanding the molecular and cellular basis of Lp(a) pathogenicity. Indeed, *LPA* is only present in Old World monkeys and apes and humans. Of note, the Old World species all lack a functional LBS in KIV10 preventing covalent OxPL addition to this kringle. Work on transgenic mice expressing human Lp(a) is progressing (Yeang et al. 2016), and ultimately should complement the significant insights that are being made on probing the effect of Lp(a) on human vascular and valve interstitial cell phenotypes (Fig. 10.2).

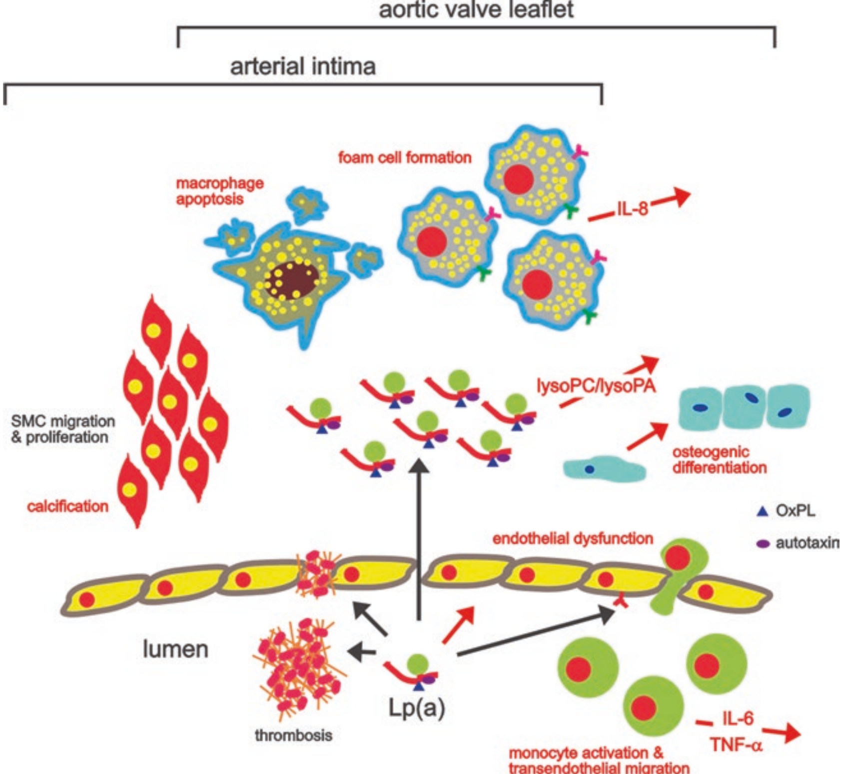

Fig. 10.2 Overlapping pathogenic mechanisms of Lp(a) in atherosclerosis and calcific aortic valve disease. There are several common mechanisms mediated by Lp(a) in the two disorders. Compromised endothelial cell function leads to barrier permeability, infiltration of Lp(a) and monocytes, expression of endothelial cell surface receptors for monocytes, and mural thromboses. Lp(a) activates monocytes leading to cytokine secretion and enhanced potential for transendothelial migration. Within the vessel wall or valve, Lp(a) promotes macrophage foam cell formation and macrophage apoptosis, as well as stimulating the release of proinflammatory cytokines such as interleukin-8 (IL-8) from macrophages. Lp(a) promotes smooth muscle cell (SMC) migration and proliferation in the arterial intima. Lp(a) also promotes calcification of SMC in the atrial intima and osteogenic differentiation and calcification of valve interstitial cells. Many functions of Lp(a), indicated in red, are mediated by its bound oxidized phospholipid (OxPL). Lp(a) also transports the phospholipase D enzyme autotaxin into the aortic valve leaflet, where it catalyzed generation of the highly proinflammatory lysophosphatidic acid (lysoPA) using lysophosphatidylcholine (lysoPC) as a substrate. The OxPL on Lp(a) thus helps to explain why elevated Lp(a) is a causal risk factor for both atherothrombosis and aortic stenosis, despite the disease processes underlying each of these disorders being distinct. *TNF-α* tumor necrosis factor-α

Effect of Lp(a) on Vascular and Immune Cell Phenotype

Effects of Lp(a) on Vascular Endothelium

The vascular endothelial cell layer is critical in maintaining a nonpermeable barrier that protects the vessel wall from exposure to blood contents. As such, there have been a number of studies aimed at determining the effect of Lp(a) on endothelial function. Lp(a) deposition in the intimal layer of the arterial wall was reported nearly 30 years ago and suggested that Lp(a) can cross the endothelial cell layer and be preferentially retained in this milieu compared to LDL (Rath et al. 1989). In 2004, it was reported that the apo(a) component of Lp(a) elicits a dramatic rearrangement of the actin cytoskeleton characterized by increased central actin stress fiber formation, redistribution of focal adhesions, and VE-cadherin disruption; these effects are a consequence of apo(a)-mediated activation of the Rho-Rho kinase signaling pathway leading to increased myosin light chain phosphorylation (Pellegrino et al. 2004). A subsequent study showed that these effects are the product of increased phosphorylation of the myosin phosphatase regulatory subunit and hence inhibition of myosin phosphatase activity, that Lp(a) and apo(a) resulted in enhanced EC permeability, and that the KIV10 sLBS was required for these effects (Cho et al. 2008). Therefore, increasing EC permeability represents a mechanism by which Lp(a) can elicit a program of EC dysfunction in early atherosclerosis, facilitating deposition of Lp(a) and LDL in the vessel wall (Fig. 10.2). In a follow-up study, enhanced prostaglandin E2 synthesis and secretion were observed when cultured HUVECs were treated with apo(a) as a result of stimulation of β-catenin nuclear translocation and increased cyclooxygenase activity (Cho et al. 2013). Lp(a) and apo(a) were shown to activate a phosphatidylinositol-3 kinase and Akt-dependent pathway that resulted in phosphorylation and inhibition of GSK3β to promote β-catenin translocation; once again, these effects were attributable to the KIV10 domain of apo(a) (Cho et al. 2013).

Early studies demonstrated the ability of Lp(a) to elicit a proinflammatory response in HUVECS through enhanced expression of E-Selectin, VCAM1, and ICAM1 (Fig. 10.2) (Takami et al. 1998; Allen et al. 1998). The apo(a) component of Lp(a) binds to the β2-integrin Mac-1 in a lysine-dependent manner; this in turn promotes the adhesion of THP-1 monocytes to ECs and enhances their Mac-1-dependent transendothelial migration (Fig. 10.2) (Sotiriou et al. 2006). Interestingly, the Lp(a)-Mac-1 binding resulted in increased expression of tissue factor in these cells. Furthermore, the interaction between apo(a) and Mac-1 induces activation of the inflammatory transcription factor NFκB (Sotiriou et al. 2006). Taken together, these studies define a novel role for apo(a)/Lp(a) in promoting inflammatory cell recruitment, which may represent a novel mechanism for Lp(a) atherogenicity in vivo (Fig. 10.2). In a more a recent study, the oxPL on KIV10 was shown to activate human aortic endothelial cells, resulting in transendothelial migration of monocytes (Fig. 10.2) (Schnitzler et al. 2020). Transcriptome analysis of Lp(a)-stimulated ECs showed upregulation of inflammatory pathways related to monocyte adhesion and migration; these effects increased 6-phophofructo-2-kinase/fructose-2,6-biphosphatase (PFKFB)-3-mediated glycolysis and could be abolished by inhibition of PFKFB3 (Schnitzler et al. 2020).

Effects of Lp(a) on Vascular Smooth Muscle Cells

A number of early in vitro studies suggested that Lp(a) could contribute to cultured vascular SMC migration and proliferation through the ability of apo(a) to inhibit the plasmin-dependent activation of TGF-β (Fig. 10.2) (Grainger et al. 1993, 1994; O'Neil et al. 2004). More recently, the ability of OxPL on apo(a) KIV10 to stimulate the expression of Klf-4, an important factor in phenotypic switching of SMCs in atherosclerotic lesions, was attributed to the apo(a)-mediated activation of the long noncoding RNA MIAT (Fig. 10.2) (Fasolo et al. 2021). Lp(a) has also been implicated in the calcification of human aortic SMCs through Notch1-NFκB and Notch1-BMP2-Smad1/5/9 pathways (Peng et al. 2022). The Notch1-NFκB pathway resulted in increased osteopontin and inflammatory cytokine expression, while Lp(a)-mediated Notch1-BMP2-Smad1/5/9 activation also contributed to calcification of the cells. The ability of Lp(a) to increase VSMC calcification is another mechanism by which Lp(a) contributes to vascular disease (Fig. 10.2).

Lp(a) and Monocyte/Macrophage Phenotype

It is well-established that Lp(a) can contribute to macrophage foam cell formation (Fig. 10.2) (Keesler et al. 1996). However, the role of the OxPL on apo(a) in macrophage function is a relatively recent finding. The first direct evidence of the OxPL on Lp(a)/apo(a) mediating a proinflammatory response in macrophages was provided by Seimon and coworkers (Seimon et al. 2010); these investigators demonstrated that the OxPL was capable of inducing apoptosis in endoplasmic reticulum (ER)-stressed macrophages in a CD36/TLR2-dependent manner (Fig. 10.2). Lp(a)/apo(a) has also been shown to contribute to monocyte recruitment through enhancing secretion of chemokines I-309 and interleukin(IL)-8 from cultured macrophages (Fig. 10.2) (Scipione et al. 2015; Haque et al. 2000; Edelstein et al. 2003). In our own studies of the apo(a)-induced IL-8 expression in macrophages, we conclusively determined that this stimulatory effect was attributable to the covalent OxPL modification on apo(a) (Scipione et al. 2015). The apo(a)/OxPL-induced signaling cascade in our study also suggested a role for CD36/TLR2 and involved the JNK- and ERK-dependent activation of NFκB—a well-known series of molecular events in inflammatory pathways—in response to OxPL-containing apo(a). The OxPL moiety on apo(a) has also been implicated in the proinflammatory priming of human peripheral blood mononuclear cells (Fig. 10.2) (van der Valk et al. 2016). The same study used high-resolution in vivo imaging to show that monocytes from high Lp(a) individuals had a propensity of trafficking to the arterial wall, a result not seen in subjects with lower Lp(a) levels (van der Valk et al. 2016).

Lp(a) and Valve Interstitial Cell Phenotype

Recent studies have demonstrated a key role for Lp(a) in both the development and progression of aortic valve disease (Thanassoulis et al. 2013; Capoulade et al. 2015). This is likely mediated, in large part, by the ability of the OxPL component of apo(a) to modify the phenotype of valve interstitial cells to resulting in proinflammatory and pro-osteogenic responses in these cells (Fig. 10.2). In a recent study by Zheng and coworkers, treatment of VICs by Lp(a) or recombinant apo(a) resulted in osteogenic differentiation in these cells through the induction of IL6, BMP2, and RUNX2 expression (Zheng et al. 2019). The effects were attributed to the OxPL on Lp(a) and apo(a): the anti-oxPL antibody E06 blocked the effects of Lp(a) as did mutation of the KIV10 LBS which significantly reduced the effect of apo(a) (Zheng et al. 2019). OxPLs transported by Lp(a) also increase the load of reactive oxygen species in the aortic valve, loading to ROS-mediated activation of the NFκB pathway, and induction of a program of inflammatory gene expression (Bouchareb et al. 2015; Mathieu et al. 2017). Additionally, Lp(a) binds to autotaxin and delivers it to valves (Bouchareb et al. 2015; Mathieu et al. 2017); autotaxin promotes inflammation and osteogenic transdifferentiation of VICs through the production of LysoPA which in turn binds and signals through the lysoPA receptor (Fig. 10.2). Taken together, these studies suggest that Lp(a) can initiate a program of inflammation and osteoblastic differentiation in valvular interstitial cells which is a major contributing factor to AVS and CAVD. Lp(a) could also promote CAVD through promotion of valve endothelial cell dysfunction, immune cell infiltration, and foam cell formation (Fig. 10.2).

Effect of Lp(a) on Thrombosis and Thrombolysis

The homology of apo(a) and plasminogen revealed by cloning of a cDNA-encoding apo(a) immediately invited speculation of an antifibrinolytic role for Lp(a) (McLean et al. 1987). However, the decades of research that have ensued have yet to provide a definitive answer on this question (Boffa 2022). There is certainly a large body of evidence from in vitro studies pointing to the ability of Lp(a) and—especially—apo(a) to inhibit plasminogen activation and impede fibrinolysis (Fig. 10.3). The earliest studies showed that Lp(a) could compete with plasminogen for binding to fibrin, endothelial cells, and monocytes (Miles et al. 1989; Hajjar et al. 1989; Rouy et al. 1992). Subsequent studies showed that Lp(a) and/or apo(a) could inhibit lysis of fibrin clots and inhibit plasminogen activation on the surface of fibrin, fibrin degradation products, and platelets (Sangrar et al. 1995, 1997; Hancock et al. 2003; Ezratty et al. 1993). Definitive demonstration of inhibition on plasminogen activation on the cell surface was only recently provided by our group (Romagnuolo et al. 2014, 2018a, b). Apo(a) was shown to inhibit thrombolysis in rabbit jugular vein

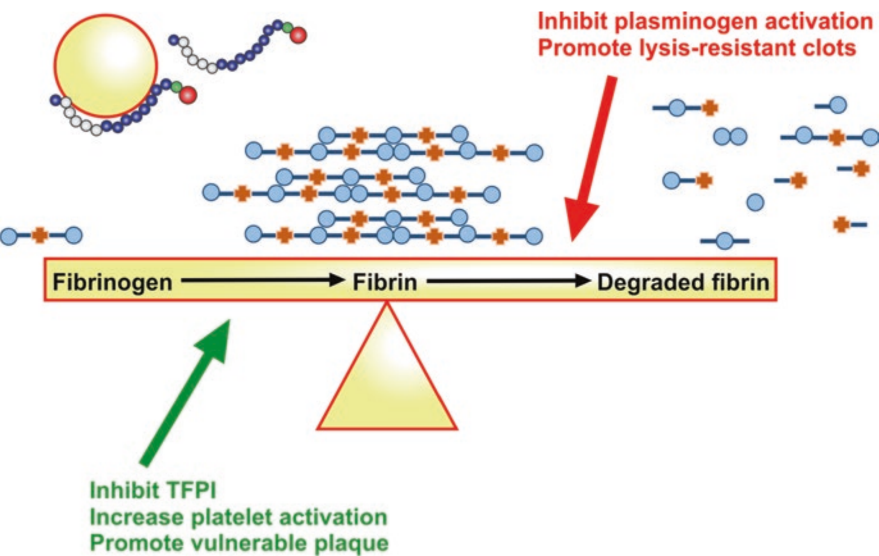

Fig. 10.3 Effects on imbalance between coagulation (formation of fibrin by thrombin cleavage of fibrinogen) and fibrinolysis (degradation of insoluble fibrin into soluble fibrin degradation products) can cause thrombosis. Lp(a) and apo(a) promote this imbalance by favoring coagulation (green mechanisms) and impeding fibrinolysis (red mechanisms). *TFPI* tissue factor pathway inhibitor

and mouse pulmonary embolism models (Biemond et al. 1997; Palabrica et al. 1995); notably, however, Lp(a) itself was not tested in these studies. Indeed, the available data from human epidemiological and genetic studies do not provide evidence for a direct antifibrinolytic/prothrombotic impact of elevated Lp(a) (Boffa 2022). Moreover, in a recent study using subjects undergoing Lp(a) lowering with antisense oligonucleotide therapy, we found that despite dramatic reductions in plasma Lp(a) concentrations, *ex vivo* plasma clot lysis time was not affected (Boffa et al. 2019). Furthermore, recombinant apo(a) had a potent antifibrinolytic effect whereas Lp(a) purified from human plasma lacked this effect (Boffa et al. 2019). Against this backdrop, we summarize below the clinical evidence with respect to Lp(a) and thrombosis and thrombolysis, and we outline areas for future studies of this issue.

Is There Direct Evidence That Lp(a) Inhibits Fibrinolysis?

Earlier observational studies provided mixed results concerning whether elevated Lp(a) is a risk factor for the development of VTE (Boffa and Koschinsky 2016). This is notable in the sense that similar studies of atherosclerotic cardiovascular disease (ASCVD) quite consistently showed elevated Lp(a) to be an independent

risk factor. With the advent of genetic approaches to study the association of genetically elevated Lp(a) with disease—including Mendelian randomization studies—the opportunity to assess a causal role for elevated Lp(a) in VTE and to eliminate confounding variables has arisen. Several large studies using genetic approaches have been published. All showed that genetically inherited elevated Lp(a) or genetical markers of high Lp(a) were not significant predictors of VTE (Kamstrup et al. 2012; Helgadottir et al. 2012; Danik et al. 2013; Larsson et al. 2020). Importantly, in several of these studies, a causal role for elevated Lp(a) in the development of atherothrombotic disorders was observed in the same population (Kamstrup et al. 2012; Helgadottir et al. 2012; Larsson et al. 2020). However, a posthoc observational study of one of these populations found that extremely high Lp(a) levels (≥95th percentile) were significantly associated with VTE (Kamstrup et al. 2012). A recent retrospective study of pulmonary embolism patients found no correlation between Lp(a) levels and the severity of pulmonary embolism (Gressenberger et al. 2022).

The general lack of association between elevated Lp(a) and risk for VTE (except for extremely high Lp(a) concentrations) is consistent with a minimal or absent antifibrinolytic ability of Lp(a). Venous thrombi are fibrin- and erythrocyte-rich and form as a consequence of blood stasis, hypercoagulability, and endothelial damage. Thrombi in the arterial tree are platelet-rich and fibrin-poor, and generally form as a sequela of atherosclerotic plaque erosion or rupture. Thus, mechanistic implications of associations between Lp(a) levels and ASCVD endpoints are confounded by the possibility that Lp(a) may contribute to atherosclerosis, thrombosis, or both. Interestingly, a consistent observation (albeit from relatively small sample sizes) has been the association between elevated Lp(a) levels and risk of ischemic stroke in children (Nowak-Gottl et al. 1999; Strater et al. 2002; Kenet et al. 2010; Goldenberg et al. 2013). These events are frequently seen in patients with inherited dispositions toward thrombophilia such as Factor V Leiden. That the events would occur in the absence of underlying atherosclerosis are clear from the young age of the patients, and this may speak to a prothrombotic or antifibrinolytic effect of Lp(a).

Early studies examined the proposition that elevated Lp(a) could reduce the efficacy of thrombolytic therapy. Most of these occurred in the setting of acute myocardial infarction (MBewu et al. 1994; Tranchesi et al. 1991; Armstrong et al. 1990; von Hodenberg et al. 1991; Brugemann et al. 1994), although one examined ischemic stroke (Ribo et al. 2004). None of these studies showed that Lp(a) levels are a significant predictor of successful recanalization, although all of them were limited by small sample sizes (and thus few patients with high Lp(a)) and/or having sampled blood for Lp(a) measurement in the postinfarct period where the acute phase response may have increased Lp(a) levels (Boffa 2022).

Taken together, the jury is still out on whether Lp(a) inhibits fibrinolysis in vivo. Further studies in animal models may be required to assess this question, and further assessment of the impact of Lp(a) on thrombolytic therapy in the setting of ischemic stroke, deep vein thrombosis, and pulmonary embolism may be warranted.

Could Lp(a) Promote Thrombosis Indirectly?

Elevated Lp(a) is clearly and consistently associated with ASCVD events, though less so with intermediate phenotypes such as intimal-medial thickness and coronary calcium scores (Kivimaki et al. 2011; Raitakari et al. 1999; Razavi et al. 2021; Mehta et al. 2022). This can be interpreted to mean that Lp(a) plays a more important role in precipitating atherothrombotic events, rather than in promoting the underlying atherosclerotic process. Two scenarios can be contemplated.

1. Lp(a) could be promoting thrombus formation directly through an impact on the coagulation system or on platelet activation (Fig. 10.3). Little to no data on an effect of Lp(a) on coagulation have been published, although early studies showed that Lp(a) could exert a procoagulant effect by binding and inhibiting tissue factor pathway inhibitor (Fig. 10.3) (Caplice et al. 2001). We and others have shown that Lp(a) and apo(a) can impact fibrin clot structure, leading to a form resistant to lysis (Scipione et al. 2017; Skuza et al. 2019; Rowland et al. 2014) (Fig. 10.3); we also demonstrated that apo(a) could accelerate the rate of fibrin formation, which could also impact clot structure (Scipione et al. 2017). Lp(a) does bind to platelets (Ezratty et al. 1993; Martinez et al. 2001), and Lp(a) and apo(a) have been shown increase the proaggregant effect of certain agonists (such as the protease-activated receptor-1 ligand peptide SFLLRN and arachidonic acid) in washed platelets (Martinez et al. 2001; Rand et al. 1998). However, two recent studies in platelet-rich plasma showed that Lp(a) level did not predict the aggregation response to several agonists including ADP, collagen, or arachidonic acid (Salsoso et al. 2020; Kille et al. 2021).
2. Lp(a) could be promoting a "vulnerable" plaque phenotype with a greater propensity to rupture and hence cause an atherothrombotic event (Fig. 10.3). Consistent with this scenario, it was reported in a carotid ultrasound study that Lp(a) level predicted the extent of stenosis but not total plaque area (Klein et al. 2008); the extent of stenosis could be interpreted to reflect ongoing rupture and thrombus formation. The proinflammatory effects of Lp(a) owing to its OxPL could result in a larger necrotic core and/or a thinner fibrous cap, both hallmarks of rupture-prone plaques. Very little direct study of this question has been attempted. In an immunohistochemical study of human coronary atherosclerotic plaques of varying phenotypes, apo(a) immunostaining was found in proximity to oxidation-specific epitopes—such as the OxPL recognized by E06—specifically in vulnerable or ruptured plaques (van Dijk et al. 2012).

It is clear from the above that, while some evidence for Lp(a)-promoting vulnerable plaque and/or arterial thrombosis exists, more research is necessary. This will require both work in animal models, such as transgenic mice expressing human Lp(a), as well as more imaging and biomarker studies in human patients. Such research is important because it may help to stratify risk in patients with high Lp(a) to identify those who would most benefit from emerging Lp(a)-lowering therapies.

Concluding Remarks

These are exciting times in the Lp(a) field, with burgeoning interest in this causal risk factor for CVD on from both basic researchers and clinicians. The quantitative importance of elevated Lp(a) as a risk factor is now widely accepted, although at present clinical adoption of Lp(a) measurement has lagged because of a lack, currently, of Lp(a)-lowering therapies. With Phase III cardiovascular outcomes trials in progress on an antisense oligonucleotide-targeting LPA mRNA, we are potentially on the cusp of having an effective treatment for lowering Lp(a) as well as definitive proof that elevated Lp(a) is harmful. At the same time, our understanding of the pathogenic mechanisms of Lp(a) continues to expand, with the role of Lp(a) as a proinflammatory mediator emerging as a key factor. Further studies of these mechanisms may lead to an alternative therapeutic strategy to mitigate the effect of elevated Lp(a)—interference with its pathogenic effects in the vasculature.

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Chapter 11
Thrombosis, Inflammation, and Lipoprotein(a): Clinical Implications

Maya S. Safarova and Patrick M. Moriarty

Lipoprotein(a) and Homeostasis: Multiple mechanisms mediate thrombus formation, including activation of platelets, coagulation, and fibrinolysis (Fig. 11.1). This figure is a schematic summary of clot formation and propagation, highlighting the complexity of interactions between lipoprotein(a) (Lp(a)), coagulation, fibrinolytic, and inflammatory factors. Lp(a), apo(a), and its fragments can bind to the extracellular matrix of arterial and venous walls. Changing conditions of blood flow and high shear stress impact the interplay between thrombus development in the arterial and venous vessels. Elevated levels of Lp(a) mediate thrombus formation and slow plasmin generation through tissue- and urokinase-plasminogen activator (t-PA and u-PA), plasminogen activator inhibitor 1 (PAI-1), alpha 2 antiplasmin, thrombin activatable fibrinolysis inhibitor (TAFI), thrombin released from activated platelets, elastase released from polymorphonuclear leucocytes. Lp(a) can bind to fibrin. Apo(a) can inactivate tissue factor pathway inhibitor (TFPI), which augments factor VII and X activation-promoting blood coagulation. Further, *LPA* (encodes Lp(a)/apo(a)) and *PLG* (encodes plasminogen) genes are organized in a head-to-head configuration and share an intergenic region (~50,000 base pairs) on the sixth chromosome. Through an interleukin 6 (IL-6) response element -CTGGGA- of the

M. S. Safarova
Atherosclerosis and Lipid Genomics Laboratory, Mayo Clinic, Rochester, MN, USA

Department of Cardiovascular Medicine, University of Kansas Hospital and Medical Center, Kansas City, KS, USA
e-mail: msafarova@kumc.edu

P. M. Moriarty (✉)
Division of Endocrinology, Diabetes and Clinical Pharmacology, Department of Internal Medicine, University of Kansas Medical Center, Kansas City, KS, USA

Atherosclerosis and Lipoprotein Apheresis Center, University of Kansas Medical Center, Kansas City, KS, USA
e-mail: pmoriart@kumc.edu

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_11

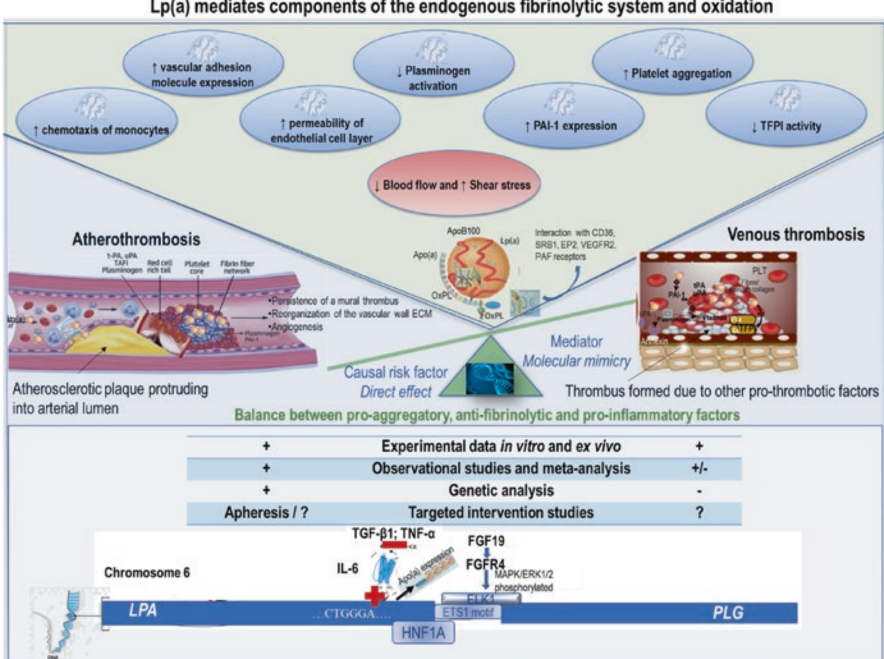

Fig. 11.1 Lp(a) mediates components of the endogenous fibrinolytic system and oxidation

LPA gene, IL-6 can induce apo(a) expression. As shown, transcription factor ELK1 mediates expression of apo(a) through the Ets domain in the *LPA* promoter; fibroblast growth factor 19 (FGF19) has inhibitory effects on apo(a) expression. The oxidized phospholipids (oxPL)-Lp(a) complex can upregulate adhesion molecules, increase secretion of chemo-attractants and cytokines, interact with various signal transduction receptors on the cell surface, and modulate binding of leukocytes to endothelial cells. Cells can recognize oxPL through scavenger receptors (SR) CD36 and SR class B type 1 (SR-B1), prostaglandin E2 receptor 2 subtype (EP2), vascular endothelial growth factor receptor 2 (VEGFR2), and the platelet-activating factor (PAF) receptor. Smaller Lp(a) isoforms have stronger association with oxPL. Oxidation of Lp(a) increases clot density. Role of Lp(a)-targeted therapies in mediating levels of pro-inflammatory, pro-thrombotic, and antifibrinolytic markers and its effects on clinical outcomes needs further investigation. In this chapter, we discuss clinical relevance and implications of pro-thrombotic and pro-inflammatory states associated with Lp(a).

Clinical Vignette

A previously healthy 11-year-old boy was admitted for a 5-day history of headaches, dizziness, and lethargy. His exam showed visual field defects and memory impairment. Magnetic resonance imaging of the brain demonstrated acute strokes in the right parietal, left cerebellar, left thalamic, and bilateral frontal lobes. An

extensive hypercoagulable, cardiac, immunological, inflammatory, and oncological work-up was unremarkable. No history of head or neck trauma was elicited. He was found to have an elevated lipoprotein(a) (Lp(a)) of 131 mg/dL (desirable level <30–50 mg/dL). Both his sister and mother had significantly elevated Lp(a). Six days after his initial hospitalization, he developed a conjugate gaze palsy and right-sided weakness due to a small left vertebral dissection associated with thrombus in the basilar and left posterior cerebellar artery. He was unable to ambulate but could follow simple commands. He underwent thrombolysis with mechanical thrombectomy allowing for near-complete arterial recanalization. Given the recurrent nature of thrombotic events with no clear inciting event, on the eighth day of hospitalization he was started on lipoprotein apheresis. Over the past 6 years, his physical capacity has improved significantly, and he returned to school and started practicing playing baseball (Moriarty et al. 2017).

A 31-year-old woman in her second trimester was hospitalized with a new onset change in mental status, right arm weakness, and subsequent focal seizures. Magnetic resonance venography revealed a superior sagittal sinus thrombosis. Four years prior, during her first pregnancy, she had another episode of cerebral venous thrombosis. She recovered with no neurological deficits and reportedly had an excellent exercise capacity prior to this event. There was no history of head trauma. Her past medical and drug history was unremarkable except for prior smoking. Her family history was unknown. Extensive work-up including autoimmune disorders and infectious etiology was negative. Initial hypercoagulopathy work-up revealed elevated fibrinogen 610 mg/dL and Lp(a) of 420 nmol/L (desirable level <75 nmol/L). Repeat coagulopathy panel in 6 months was unremarkable. Except for pregnancy, previous smoking, and elevated Lp(a), no other known risk factor for cerebral venous thrombosis was identified. She was initially treated with low molecular weight heparin. Following discussion of risks and benefits, she was started on biweekly lipoprotein apheresis. Over the course of the next 5 years, no new ischemic or thrombotic events were observed. During this time, she completed her third pregnancy with no complications while receiving regular apheresis.

Introduction

> *The point of life is to find the delicate equilibrium between dream and reality.*—Lillian Eugenia Smith

A high plasma level of Lp(a) is a causal risk factor for atherosclerotic cardiovascular disease (ASCVD) through various pathways associated with increased atherogenesis, inflammation, and thrombosis (Reyes-Soffer et al. 2022; Tsimikas 2017). Lp(a) affects endothelial function and mediates inflammation and oxidative stress, fibrinolysis, and plaque stability, leading to accelerated atherothrombosis. Such life-threatening and debilitating events as described in the above clinical vignettes motivate to continue building the foundation of understanding clinical implications of

Lp(a) in: (1) (athero)thrombosis, (2) platelet and coagulation cascade, and (3) inflammation. According to the 2018 National Heart Lung and Blood Institute (NHLBI) report, an estimated 1.4 billion people globally have Lp(a) levels ≥50 mg/mL (>100–125 nmol/L) with a prevalence ranging from 10% to 30% (Tsimikas et al. 2018). The prevalence is higher in patients with established ASCVD, calcific aortic valve disease, and chronic kidney disease (Tsimikas et al. 2018).

Lp(a) is a lipoprotein with a density between 1.06 and 1.11 g/mL that can bind to lysine-rich components of the coagulation system and several components of extracellular and subendothelial matrix of the vascular wall via its apoB and apolipoprotein(a) (apo(a)), including binding to fibronectin, fibrinogen, glycosaminoglycans, and proteoglycans (Kostner and Bihari-Varga 1990; Klezovitch et al. 1998). Lp(a) can be retained in the arterial intima, localizing preferentially to atherosclerotic plaques (Boffa and Koschinsky 2016). The low-density lipoprotein (LDL) moiety of the Lp(a) particle is covalently linked to the plasminogen-like glycoprotein apo(a) through a single disulfide link between apolipoprotein B100 Cys 3734 and apo(a) kringle IV type 9 Cys67. Lp(a) is almost completely confined to a subset of primates. It has been proposed that the duplication of the *PLG* gene evolved into the *LPA* gene (Lawn et al. 1995).

Similarities between the two genes (*LPA* and *PLG*) include 5′-flanking and untranslated regions, multiple copies of kringle IV-, a single copy kringle V-like and protease-like domains, as well as a related 3′-untranslated region (Lawn et al. 1995; McLean et al. 1987). An Lp(a)-like complex is found in hedgehogs which is thought to have evolved independently from that of humans. While individuals with low concentrations of plasma Lp(a) typically show no syndromic features or pathologic conditions, the physiological role of Lp(a) in humans is not entirely clear. An analysis of individual-level data of 112,338 UK Biobank participants demonstrated that one standard deviation of genetically lowered Lp(a) level was associated with reduction in risk of coronary heart disease (CHD) and peripheral vascular disease by 30% (odds ratio, 0.71; 95% confidence interval, 0.69–0.73 and 0.69; 0.59–0.80, respectively), ischemic stroke by 13% (0.87; 0.79–0.96), aortic valve stenosis by 37% (0.63; 0.47–0.83) (Emdin et al. 2016). These findings were reproduced with the *LPA*-rs41272114 (null allele frequency, 3%) associated with low Lp(a), providing a significant protective effect in carriers with a ~20% risk reduction in CHD (Kyriakou et al. 2014). No association of genetically predicted low Lp(a) levels with type 2 diabetes, malignancy, or venous thromboembolism (VTE) was observed in this study (Emdin et al. 2016). Current hypothesis of the evolutionary advantages of synthesizing Lp(a)-like particles includes accelerated repair of vascular lesions, tissue injuries, healing of wounds, reduced bleeding, as well as induction and participation in different forms of acute phase responses (Lippi and Guidi 2000; Brown and Goldstein 1987; Kronenberg and Utermann 2013; Caplice et al. 2001; Boffa et al. 2004; von Zychlinski et al. 2011). However, these properties can become pathogenic in the setting of increased concentrations of the lipoprotein and homeostatic imbalance.

Lp(a) and Thrombosis

It is hard to imagine that nature is only teasing us and the structural resemblance between apo(a) and plasminogen has no clinical consequences.—Michael S. Brown and Joseph L. Goldstein (1987)

Apo(a) is highly homologous to plasminogen, demonstrating antifibrinolytic activity and pro-thrombotic properties. Data suggest that the apo(a) component of Lp(a) inhibits conversion of plasminogen to plasmin by endogenous tissue plasminogen activators as well as competes with plasminogen and plasmin for binding to established fibrin clots, thus compromising clot lysis. Other potential prothrombotic effects of Lp(a) include an increase in the expression of plasminogen activator inhibitor 1 (PAI-1) (Etingin et al. 1991), inhibiting fibrinolysis, and inactivation of tissue factor pathway inhibitor (TFPI) (Caplice et al. 2001), which augments factor VII and X activation and therefore promotes blood coagulation. In a series of experiments using human plasma, Lp(a) had a higher binding affinity for TFPI compared to plasminogen with its apo(a) component precluding binding of plasminogen to TFPI. Enhanced interaction between Lp(a) and TFPI and the loss of TFPI activity was observed in the Lp(a)-rich environment within the subendothelial space of plaques (Caplice et al. 2001). Reduced affinity of Lp(a) to the vessel wall and decreased fatty streak formation was demonstrated in experiments with defective lysine binding sites of the apo(a) kringle IV type 10 (Hughes et al. 1997; Boonmark et al. 1997).

It has been proposed that Lp(a) may increase clot density (Undas et al. 2006; Scipione et al. 2017) and may skew the balance of endogenous coagulation and fibrinolysis to propagate thrombus enlargement and provide resistance to thrombolysis (Angles-Cano et al. 2001). In the context of Lp(a), different weights might need to be applied to the components of arterial and venous thrombosis driven by platelet aggregation (white thrombus) and entrapment of erythrocytes by fibrin (red thrombus), respectively. In patients with pre-existing chronic inflammatory conditions (i.e., rheumatoid arthritis, vasculitis) and in the presence of other procoagulable states (i.e., polycythemia vera, antiphospholipid syndrome, factor V Leiden heterozygosity, protein C deficiency, etc.) (Espinosa et al. 2001; Nowak-Gottl et al. 1997), elevated Lp(a) has been shown to promote both arterial and venous thrombotic events. In a retrospective analysis of the Hematology Clinic referrals of females younger than 50 years with deep venous thrombosis or pulmonary embolism who had negative hypercoagulable panel, Lp(a) was elevated at >75 nmol/L in 57% of cases (Nguyen et al. 2018). Most of the screened patients with high Lp(a) did not have any other clinical risk factors for thrombosis (Nguyen et al. 2018). In younger individuals aged <50 years, the risk for recurrent cerebral venous thrombosis was fourfold (odds ratio, 3.9; 1.23–12.4) higher in patients with Lp(a) levels above 30 mg/dL during a median follow-up of 4.4 years, especially following discontinuation of anticoagulation (Skuza et al. 2019). No difference in thrombophilia risk factors among the groups was reported.

Observational and genetic studies have demonstrated a strong association between Lp(a) levels and such atherosclerotic traits as coronary and peripheral artery disease, abdominal aortic aneurysm, ischemic stroke, aortic valve stenosis (discussed in other chapters of this book). Earlier onset of coronary artery disease was observed in the carriers of *LPA*-rs10455872 and *LPA*-rs3798220 associated with elevated Lp(a) levels (Helgadottir et al. 2012). Increased rates of paravalvular leaks were reported in patients with high Lp(a) treated with transcatheter aortic valve replacement (Ma et al. 2019). Lp(a) is a significant predictor of resistance to endogenous thrombolysis in the early phase of acute myocardial infarction (Kim et al. 2008), mediating insufficient fibrinolysis in the infarct-related arteries early post thrombolytic administration (Brugemann et al. 1994). Using a Mendelian randomization-phenome-wide association approach in individuals of European and African ancestry with genetically predicted elevated Lp(a) levels, a 30% increase in the risk of arterial thromboembolic disease was observed with no significant association with any VTE phenotypes (Satterfield et al. 2021). An increase in the risk of atrial fibrillation (hazard ratio, 1.07; 1.04–1.10) for every one standard deviation increase in genetically predicted Lp(a) levels was demonstrated in this cohort (Satterfield et al. 2021). In a total of 367,586 unrelated UK Biobank participants of the European-descent, there was no significant association with VTE (pulmonary embolism or deep vein thrombosis) per genetically predicted 50-mg/dL increase in Lp(a) levels (Larsson et al. 2020).

In relation to venous thrombosis and Lp(a), there are conflicting data. The likelihood of VTE in the general population was shown to increase with older age, smoking, and obesity (Gregson et al. 2019; Heit et al. 2012). Positive family history has been associated with doubling of the risk of incident venous thrombosis; risk was up to fourfold higher when more than one relative was affected, regardless of the presence of other risk factors (Bezemer et al. 2009). In a number of observational studies, elevated Lp(a) levels were shown to be elevated in 20–36% of individuals diagnosed with VTE (Nowak-Gottl et al. 1997; von Depka et al. 2000; Sofi et al. 2007). No significant difference in the Lp(a) levels was seen in patients with recurrent unprovoked VTE (Rodger et al. 2010). However, a meta-analysis of 17,688 individuals showed a 2.4-fold increase in the odds of retinal vein thrombosis (odds ratio, 2.4, 1.7–3.3) in patients with elevated Lp(a) levels (Paciullo et al. 2021). Lp(a) was associated with a 6.5-fold (4.46–9.55) increase in the odds of incident arterial ischemic stroke and cerebral venous sinus thrombosis events among other genetic factors in 4563 children and adolescents aged less than 18 years (Kenet et al. 2010). In a meta-analysis of 14 studies with 2824 VTE patients and 11,187 healthy controls, elevated Lp(a) was associated with a 1.6-fold (1.36–1.79) increase in the risk of VTE (a large amount of heterogeneity was reported, $I^2 = 77\%$) (Dentali et al. 2017). In a prospective cohort study of individuals aged <21 years, Lp(a)-mediated impaired fibrinolysis was thought to contribute to substantially increased risk of recurrent arterial ischemic stroke for race-specific Lp(a) levels above the 90th percentile with an odds ratio of 14.0 (1.0–184), and apo(a) isoform size less than 10th percentile with an odds ratio of 12.8 (1.61–101) (Goldenberg et al. 2013).

In contrast, when applying a Mendelian randomization study design in the Danish adult population, only extreme levels of Lp(a) (total n = 14,783) and very small apo(a) isoforms (total n = 38,753) were associated with a 1.7-fold (1.2–2.3) and 1.3-fold (1.0–1.7) increase in the likelihood of venous thrombosis (deep vein thrombosis and pulmonary embolism), respectively (Kamstrup et al. 2012). In the same population, the risk of atherothrombosis (myocardial infarction) was associated with both Lp(a) tertiles and KIV-2 repeat tertiles (Kamstrup et al. 2012). Among 9330 white men and women from the general population during a 10-year follow-up, a significant increase in the risk of myocardial infarction was seen starting at Lp(a) levels of 30 mg/dL. The risk incrementally grew with increases in Lp(a) levels and was estimated at 3.6-fold (1.7–7.7) for individuals with Lp(a) ≥95th percentile (≥120 mg/dL) (Kamstrup et al. 2008). When this population was expanded to 53,908 individuals with a total of 2501 VTE events, there was a 30% (odds ratio, 1.33; 1.06–1.69) increase in the risk of VTE among patients with Lp(a) above 100 mg/dL (Nordestgaard et al. 2010). In a Japanese population of 10,494 individuals, low Lp(a) levels (<10 mg/dL) were found to be associated with a higher frequency of hemorrhagic strokes with no difference in ischemic strokes (Ishikawa et al. 2013). It is plausible that this discordance in the reported data could be attributed to: (1) the lack of power to detect significant associations as well as low enrichment with extremely high Lp(a) levels, and (2) confounding with the presence of inflammatory and pro-thrombotic conditions affecting outcomes but not registered in the large datasets resulting in reverse causation when the exposure-disease process is reversed.

Lp(a), Platelets, and Coagulation

Lp(a) has been shown to inhibit endogenous fibrinolysis through: (1) competitive inhibition of pericellular plasminogen activation on vascular and blood cells mediated by tissue plasminogen activator (tPA), (2) mediation of plasminogen binding to platelets, (3) competition with plasminogen for binding to fibrin, mononuclear cells, annexin II on endothelial cells, (4) inhibition of fibrinogen binding to platelets activated with platelet-activating factor (PAF) (Loscalzo et al. 1990; Edelberg et al. 1989; Hajjar et al. 1989; Simon et al. 1991; Ezratty et al. 1993; Edelberg and Pizzo 1995; Moliterno et al. 1993; Romagnuolo et al. 2014). In mice resistant to tPA-mediated thrombolysis, apo(a) was reported to reduce clot lysis *in vivo* (Palabrica et al. 1995). Clot lysis was attenuated in the setting of apo(a) transgene when compared to their sex-matched normal littermates. Experiments with removal of kringle V and the lysine binding site in kringle IV type 10, respectively, negated and substantially reduced the inhibitory effect of apo(a) (Romagnuolo et al. 2014). In cultured endothelial cells, Lp(a) was shown to induce rearrangements of actin filaments (Pellegrino et al. 2004) and upregulate the expression of PAI-1, thereby reducing the amount of tPA available for plasminogen activation (Levin et al. 1994). Lp(a)-mediated plasmin recognition of fibrin clots was demonstrated in experiments

showing that apo(a) upregulated α2-antiplasmin (Edelberg and Pizzo 1992), thereby impairing fibrinolysis.

In the presence of Lp(a), washed human platelets demonstrate significant enhancement of aggregation and release of granule contents (Rand et al. 1998). In direct binding experiments, specific and reversible binding of Lp(a) to platelets was observed (Ezratty et al. 1993). On the other hand, activation of platelets with adenosine diphosphate (ADP) halted Lp(a) binding capacity. Further, in a dose-dependent manner, Lp(a) was shown to inhibit PAF-induced platelet activation as well as primary and secondary platelet aggregation induced by ADP and calcium. When apo(a) was completely removed from the Lp(a) particle, its inhibitory effect on PAC-1 (a mouse monoclonal antibody indicative of platelet activation) binding to activated platelets significantly enhanced the antiaggregatory effects in comparison with the "unreduced" Lp(a) (Tsironis et al. 2004).

There is controversy surrounding apo(a) isoform size dependent versus independent inhibition of plasminogen activation by Lp(a). In the clinical setting, it has been demonstrated that the size of apo(a) isoforms was inversely associated with an up to eightfold increase in the risk of thromboembolic events (Espinosa et al. 2001; Falco et al. 1998). In a case-control study, copy number variation of *LPA* KIV-2 was an independent determinant of VTE (Sticchi et al. 2016). The KIV type 2 repeat number was significantly lower in patients with VTE than in healthy controls, including an observed higher frequency of the KIV-2 repeat number of less than 8 (Sticchi et al. 2016). Levels of Lp(a) may vary up to 200-fold for a given apo(a) isoform (Perombelon et al. 1994). Single nucleotide polymorphisms mapped to *LPA* and KIV-2 copy number have been shown to provide complementary information, explaining the variation in plasma Lp(a) concentrations (Lanktree et al. 2010). Thus, while some research has shown that small size apo(a) isoforms display higher affinity to bind fibrin (Angles-Cano et al. 2001), there are data demonstrating a lack of association between the *LPA* score comprising rs10455872 and rs3798220 variants (Helgadottir et al. 2012; Danik et al. 2013), number of KIV type 2 repeats, and the level of plasminogen inhibition (Romagnuolo et al. 2014; Hancock et al. 2003), highlighting a need for further research in this area.

Lp(a) and Inflammation

Lp(a) is the preferential lipoprotein carrier for oxidized phospholipids (OxPL), pro-atherogenic and pro-inflammatory markers. *In vitro* experiments demonstrated that products of oxidation make fibrin clot less permeable (Hoffman 2008), another mechanism by which Lp(a) may be affecting clot lysis. In individuals with elevated Lp(a) levels (>50 mg/dL), a local increase in the arterial wall inflammation *in vivo*, enhanced peripheral blood mononuclear cells trafficking as well as transendothelial migration and accumulation in the arterial wall with and without plaquing was noted (van der Valk et al. 2016). Further, it was shown that patients with familial hypercholesterolemia (average LDL-C, 236 mg/dL) and elevated Lp(a) levels

(range, 43–401 nmol/L) elicit markedly increased local arterial wall inflammation as compared to healthy control subjects (van Wijk et al. 2014). Following lipoprotein apheresis, a significant reduction of the arterial wall inflammation estimated using the target-to-background ratio of a fluorodeoxyglucose (FDG) uptake on the PET/CT scan was demonstrated (van Wijk et al. 2014). This anti-inflammatory effect was observed after a single session of apheresis. Milder reduction in the Lp(a) levels with PCSK9 inhibitors did not show any significant change in target-to-background ratio of the index arterial vessel as compared to placebo (Stiekema et al. 2019).

Mechanism by which cells recognize oxPL include recognition and interactions with the cell receptors such as CD36, SRB1, EP2, VEGFR2, Toll-like receptor-4, and the PAF receptors (Zimman et al. 2007; Berliner et al. 2009). In the middle-aged patients with type 2 diabetes, oxidative stress enhanced pro-thrombotic state by unfavorably affecting the fibrin network structure and impairing fibrin clot susceptibility to lysis regardless of diabetes severity and duration (Lados-Krupa et al. 2015). Oxidized apoB100-containing lipoproteins were independently associated with the clot lysis time in diabetes, suggesting that oxidized forms might directly impair plasmin-mediated fibrin degradation (Lados-Krupa et al. 2015). An aggregation of Lp(a) particles on the surface of the aortic smooth muscle cells was observed in the presence lipoprotein lipase and sphingomyelinase (Tabas et al. 1993). In the same series of experiments, coincubation of Lp(a)-coated smooth muscle cells with macrophages resulted in formation of lipid-laden macrophages (Tabas et al. 1993). The role of macrophages in Lp(a) pathophysiology is discussed in Chap. 10.

Several mechanisms of Lp(a)-mediated plaque instability have been described, including increased production of interleukin-8 (IL-8). IL-8 among other effects disinhibits matrix metalloproteinases (Moreau et al. 1999; Ezhov et al. 2019), which increases the intensity of plaque inflammation and likelihood of plaque rupture. Oxidized Lp(a) fractions induce a dose-dependent reduction of nitric oxide synthase expression and, as a result, reduce nitric oxide production (Moeslinger et al. 2006), thereby adversely affecting vascular homeostasis. In an assessment of vasomotor response to acetylcholine, an association between elevated Lp(a) levels and impaired endothelium-dependent vasodilatation in coronary arteries with and without angiographically detectable atherosclerotic lesions was observed (Tsurumi et al. 1995). In a dose-dependent manner, Lp(a) promotes intercellular cell adhesion molecule-1 (ICAM1) expression (Takami et al. 1998), upregulation of which has a key role in the inflammatory response (Libby et al. 2011). Proteomic analysis of Lp(a) revealed its association with the histidine-rich glycoprotein (HRG) known to interact with heparin, plasminogen, fibrinogen, and complement components (von Zychlinski et al. 2011). Further there was a strong signal for the C3 complement component in the Lp(a) position in the plasma protein fraction, highlighting an association of C3 with the Lp(a) particle (von Zychlinski et al. 2011; Garcia-Arguinzonis et al. 2021).

In critically ill patients with COVID-19 infection, treatment with monoclonal antibodies against IL-6 receptor (such as tocilizumab and sarilumab) was shown to

improve survival (Investigators et al. 2021). In patients with underlying pro-inflammatory conditions (rheumatoid arthritis, COVID-19 infection), tocilizumab significantly reduced Lp(a) levels and increased LDL-C levels by affecting LDL-receptor expression in hepatocytes (Strang et al. 2013; Pierini et al. 2021; Miller et al. 2015). The sequence of the *LPA* gene contains several IL-6-responsive elements that enhance transcription of the gene (Wade et al. 1993). *In vitro* studies have shown that Lp(a) stimulates growth of endothelial and smooth muscle cells, including through inhibition of transforming growth factor-β activation (Grainger et al. 1993). In human hepatocytes, tocilizumab inhibited IL-6-induced *LPA* mRNA and protein expression whereas monoclonal inhibition of TNF-α with adalimumab did not affect Lp(a) levels (Miller et al. 2015).

Individuals infected with COVID-19 demonstrate a wide range of symptoms—from mild symptoms to severe illness. Since 2020, there is ongoing research on the role of Lp(a) in patients with COVID-19 infection. For instance, in participants of the UK Biobank, there was no difference in the Lp(a) distribution between individuals who tested positive for SARS-CoV-2 (n = 13,588) and population controls (average Lp(a) level was ~20 nmol/L in both groups) (Di Maio et al. 2021). In this predominantly white population, each 25 nmol/L increase in the Lp(a) levels was associated with a 4% and 7% increase in the risk of ischemic heart disease in COVID-19-negative (n = 435,104) and COVID-positive (n = 6937) individuals, respectively. There was a significant interaction between SARS-CoV-2-positive status and Lp(a) levels with a 2.2-fold increase in the risk in patients with Lp(a) >95th percentile (>220 nmol/L) compared to the bottom 20% (<6 nmol/L) of the Lp(a) distribution. This association between Lp(a) and ischemic heart disease was most pronounced in the younger age group. The incidence of thromboembolic events was an eight-fold higher in patients requiring an intensive care unit stay compared to SARS-CoV-2-positive patients without ICU treatment, and a 34-fold higher when compared to the population controls. There was no significant association between Lp(a) levels or *LPA* genetic scores and the risk of thromboembolic events among the SARS-CoV-2-positive patients and the population controls (Di Maio et al. 2021). This heterogeneity in the findings for the role of Lp(a) in patients with COVID-19 infection can be attributed to the intrinsic differences in selected populations with differences in disease severity, treatment impact for COVID-19-positive patients, and changes in Lp(a) during the acute phase of the disease. Further, although human apo(a) can bind to substrates shared by its plasminogen homologue, it does not innately perform as an activable plasmin-like protease, potentially adding another confounding layer to conflicting results with VTE outcomes.

Future Directions, Relevance, and Conclusions

Medicine is the art of addition and subtraction. The subtraction of all that is excessive, and the addition of all that is missing. And he who might be the best at doing this — will be the best doctor.—Hippocrates Asclepiades.

Antiplatelet Agents and Anticoagulation

Among carriers of the *LPA*-rs3798220 variant (median Lp(a) level >80 mg/dL) in the Women's Health Study, aspirin intake was associated with a 56% (hazard ratio, 0.44; 0.20–0.94) reduction in the risk of major cardiovascular events, including myocardial infarction, ischemic stroke, and cardiovascular death (Chasman et al. 2009). After a median of 9.9 years, there was a twofold decrease in the absolute risk in the placebo group: the event rate was 2.14% (0.81–3.45%) in the aspirin group and 4.83% (2.74–6.87%) with placebo (Chasman et al. 2009). In Japanese patients, treatment with aspirin was associated with a significant reduction in Lp(a) levels (Akaike et al. 2002). These findings were reproduced in the South Asian population aged 21–60 years with aspirin significantly reducing Lp(a) levels (Ranga et al. 2007). Supporting experimental data in mice suggested that aspirin can suppress apo(a) mRNA expression (Kagawa et al. 1999). In healthy individuals, lower Lp(a) values were measured in EDTA-treated plasma, citrated, and heparinized plasma as compared to the serum (Lippi et al. 1996). However, there are no studies investigating effects of anticoagulation on Lp(a).

Apo-B Lowering

In patients with CHD with on-statin LDL above 130 mg/dL, elevated Lp(a) levels >80th percentile additionally increased the risk of recurrent ischemic events by 40% (odds ratio, 1.40; 1.15–1.71) (O'Donoghue et al. 2014). In a meta-analysis including primary and secondary ASCVD prevention randomized clinical trials similar odds ratios (1.43; 1.15–1.76) were observed in statin-treated patients with Lp(a) levels >50 mg/dL (Willeit et al. 2018). In the primary prevention JUPITER trial (Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin) independent of the LDL-C levels, for each standard deviation change in Lp(a) while on a statin there was a 27% (hazard ratio, 1.27; 1.01–1.59) increase in the relative risk of incident ASCVD (Khera et al. 2014). Among 25,096 participants in the secondary prevention FOURIER trial (Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk), patients with higher baseline Lp(a) (>120 nmol/L) had a 25% relative risk reduction (0.64–0.88) and a 2.4% absolute risk reduction with PCSK9 inhibition, translating into an estimated number needed to treat of 41 to prevent one fatal or non-fatal myocardial infarction or urgent revascularization (O'Donoghue et al. 2019). There is ample evidence that selective lipoprotein apheresis improves clinical outcomes related to atherothrombosis in patients with elevated Lp(a) (Jaeger et al. 2009; Leebmann et al. 2013; Safarova et al. 2013). Lipoprotein apheresis results in greater than 60% reduction of apoB-containing lipoproteins following a single apheresis procedure. It has been shown to improve blood rheology, rapidly and efficiently reduce Lp(a) levels and inflammatory markers, including IL-6 and oxPL (Moriarty 2015). In patients with CHD, familial hypercholesterolemia, and elevated Lp(a) levels, treatment with lipoprotein apheresis demonstrated a significant downregulation of

mRNA expression for IL-1α, IL-6, and TNF-α (Stefanutti et al. 2017). Since 2020, the US Food and Drug Administration (FDA) approved criteria for apheresis in secondary prevention of ASCVD with LDL-C levels >100 mg/dL and Lp(a) >60 mg/dL on maximally tolerated lipid-lowering therapy (Nugent et al. 2020).

The importance of assessing Lp(a) in patients in the primary prevention setting as well as in those with prior thrombotic events relates to its ability to identify subjects at increased risk, and the potential to modulate impaired fibrinolysis and inflammation and as a result, improve outcomes. To date, extensive evidence exists in support of a causal association of elevated Lp(a) levels with the risk of atherosclerosis development and progression in different vascular beds. Likely, the presence of the atherosclerotic plaque is in fact an inciting event for Lp(a) to promote thrombus formation in the unstable milieu. Current data suggest that except for Lp(a) levels ≥90–95th percentile, small isoforms (KIV-2 repeats <10–6th percentile) and in individuals with existing pro-inflammatory and pro-thrombotic conditions, Lp(a) does not initiate venous thrombus formation, but rather contributes to its propagation and density. Lp(a) has a complex genetic architecture. For instance, studies within or outside the *LPA* gene region demonstrated that in carriers of the Lp(a)-raising genetic variants (i.e., rs10455872), the presence of a rare missense rs41267813[A] variant was associated with substantially lower Lp(a) concentrations (Said et al. 2021), potentially offering a protective effect against CHD.

Discovery of new mechanisms elucidating pathways affecting interplay between Lp(a) and acute thrombotic events will continue to provide potential therapeutic targets addressing current gaps in residual risk. Genetic studies evaluating *LPA* interaction using functional studies and its clinical relevance on the clinical outcomes involving diverse cohorts are needed. Further research is warranted to assess differences in the markers of the coagulation system activation and platelet activation before and after Lp(a)-targeted treatment to address pathophysiological relevance of pro-thrombotic and atherogenic properties of Lp(a) in humans. Studies investigating effects of aspirin in primary prevention and other antiplatelet agents in secondary prevention in patients with elevated Lp(a) are needed. Polygenic risk scores can assist in unraveling the interplay between thrombosis and inflammation in patients with elevated Lp(a), especially when tested in racially diverse populations (Table 11.1).

- Elevated Lp(a) promotes arterial and venous thrombosis.
- Similar to plasminogen, apo(a) induces thrombotic events. Antifibrinolytic effects are enhanced by pro-thrombotic properties in individuals with very high Lp(a) levels, small apo(a) isoforms, and underlying inflammatory and pro-thrombotic states.
- Similar to LDL, Lp(a) is a subject to oxidative modification, enhancing the thrombo-inflammatory response.
- High Lp(a) levels are causal for the risk of atherothrombosis.
- Higher Lp(a) levels are seen in patients with acute VTE.
- Clinical trials focusing on Lp(a)-targeted therapies addressing residual risk of high Lp(a) are on the way.

Table 11.1 Proposed research questions to bridge gaps in knowledge of clinical relevance of elevated Lp(a) in atherothrombosis and venous thrombosis

Design	Proposed study question
Novel experimental models Functional studies	Mechanisms and pathways beyond plasminogen activation and tPA-mediated fibrinolysis affecting interaction between *high* Lp(a), acute thrombosis, and unstable atherosclerotic lesion development.
Biomarker research	Markers of the coagulation system activation, fibrinolytic potential, and platelet activation before and after Lp(a)-targeted treatment, with and without other conditions precipitating thrombosis.
	Assessment of anti-inflammatory effects of Lp(a)-lowering therapies on vulnerable plaques identified using novel imaging modalities
Genetic studies	Clinical impact of genetic architecture of elevated Lp(a) on outcomes in cohorts with diverse ethnic/racial background.
Clinical/pragmatic trials	Effects of aspirin in primary prevention of ASCVD in individuals with elevated Lp(a).
	Effects of dual antiplatelet agents/more potent antiplatelet agents in secondary prevention of ASCVD in patients with elevated Lp(a).
	Role of elevated Lp(a) in determining duration of anticoagulation in patients with VTE.
	Role of elevated Lp(a) in addressing the risk of stroke in patients with atrial fibrillation.
	Assessing the role of Lp(a) and targeted treatment in patients undergoing aortic valve replacement.
Clinical trials/ outcome research	Lp(a)-targeted therapies and prognosis in patients with elevated Lp(a), identifying race-specific therapeutic targets

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Chapter 12
The Kidney Is the Heart of the Organs: Its Role in Lp(a) Physiology and Pathophysiology

Hans Dieplinger

Introduction

Lipoprotein(a) [Lp(a)] is a complex lipoprotein particle that is independently associated with atherosclerotic disease (Kronenberg and Utermann 2013). Epidemiological and genetic studies demonstrate that elevated plasma Lp(a) concentrations are highly genetically determined and dose-dependently increase causally the risk of cardiovascular events (Arsenault and Kamstrup 2022; Coassin and Kronenberg 2022; Kronenberg et al. 2022). The non-genetically elevated Lp(a) concentrations in patients with various chronic kidney diseases (CKD) suggest a role of the human kidney in Lp(a) metabolism (Kronenberg et al. 1996). This review summarizes the experimental in vitro and in vivo evidence for a role of the kidney in Lp(a) physiology and pathophysiology and aims to combine them into a clinical picture for various groups of CKD patients.

Definition of Lipoprotein(a)

Lp(a) consists of an LDL-sized particle covalently linked to the glycoprotein apo(a). Plasma Lp(a) concentrations vary widely between individuals, from hardly measurable to >200 mg/dL, and have a highly skewed distribution in most ethnic populations. The *LPA* gene encodes apo(a) on chromosome 6 and accounts for more than

The original version of the chapter has been revised. A correction to this chapter can be found at https://doi.org/10.1007/978-3-031-24575-6_26

H. Dieplinger (✉)
Department of Genetics, Institute of Genetic Epidemiology, Medical University of Innsbruck, Innsbruck, Austria
e-mail: hans.dieplinger@i-med.ac.at

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2023, corrected publication 2023
K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_12

90% of the variation in plasma concentrations (Kronenberg and Utermann 2013; Utermann 1989). *LPA* alleles contain a variable number of exons pairs encoding the plasminogen-like kringle IV (KIV) domains (McLean et al. 1987). This results in differently sized apo(a) isoforms, ranging from 300 to 800 kDa, corresponding to the presence of 11 to >50 KIV domains (Koschinsky et al. 1990; Lackner et al. 1993; Utermann et al. 1987). For practical purposes, apo(a) isoforms with ≤22 KIV repeats are defined as low molecular weight (LMW) isoforms, those with >22 repeats as high molecular weight (HMW) isoforms (Kronenberg and Utermann 2013).

Epidemiological and genetic studies have suggested that increased Lp(a) plasma concentrations are causally associated with coronary heart and aortic valve calcification (Arsenault and Kamstrup 2022; Coassin and Kronenberg 2022; Erqou et al. 2009; Nordestgaard and Langsted 2016; Sandholzer et al. 1992; Thanassoulis 2016). As a consequence, Lp(a) is a potential pharmacological target for which treatments are currently under development.

Metabolism of Lipoprotein(a)

Since the discovery of Lp(a) 60 years ago, tremendous effort has been invested in the elucidation of its molecular, cellular, and metabolic pathways. Despite an overwhelming body of experimental evidence from various in vitro and in vivo systems, the metabolism of this enigmatic lipoprotein still remains poorly understood (Chemello et al. 2022).

Synthesis, Assembly, and Secretion

Apo(a) is exclusively synthesized in hepatocytes (Kraft et al. 1989) and undergoes post-translational modifications, including the formation of three disulfide bonds within each kringle motif as well as substantial N-glycosylations. The residence time of apo(a) isoforms in the endoplasmatic reticulum is proportional to their number of KIV_2 domains (Brunner et al. 1996; White et al. 1994). Large apo(a) isoforms are more susceptible to degradation in the intracellular proteosome (White et al. 1999), explaining the (on average) higher circulating plasma Lp(a) concentrations in carriers of small apo(a) isoforms (Utermann et al. 1987). The availability of apoB-100 could be rate limiting for the assembly of Lp(a), as concluded by studies in patients with abetalipoproteinemia (Menzel et al. 1990).

Several earlier studies suggested an extracellular or cell-surface-associated assembly of Lp(a) assembly following hepatic secretion of apo(a) (Chiesa et al. 1992; McCormick et al. 1994; White and Lanford 1994; Wilkinson et al. 1994). This has, however, been challenged by studies showing that apo(a)-apoB100 complexes can be detected intracellularly (Bonen et al. 1997) and several in vivo kinetic studies in humans attempting to address the unanswered questions regarding the assembly and secretion of Lp(a) (Reyes-Soffer et al. 2017).

Kinetic studies in vivo using stable isotope tracers and compartmental modeling demonstrated that in individuals with a wide range of plasma Lp(a) concentrations, the isotopic tracer curves for Lp(a)-apoB-100 and Lp(a)-apo(a) were essentially identical, with similar contour and area-under-curve suggesting intracellular assembly of Lp(a) (Watts et al. 2018). This notion was confirmed in a further in vivo study in healthy individuals (Frischmann et al. 2012) and another kinetic study of statin-treated patients with elevated and normal Lp(a) concentrations (Ma et al. 2019).

These kinetic data therefore generally support an intracellular assembly of Lp(a) suggesting that newly synthesized Lp(a)-apoB-100 and Lp(a)-apo(a) are secreted as a holoparticle with coupled apo(a) and apoB100 residence times in the circulation. Controversial results of an extracellular Lp(a) assembly are possibly due to inappropriate cellular or animal models.

Clearance and Catabolism

The mechanisms of Lp(a) clearance from the circulation also remain unclear and controversial (McCormick and Schneider 2019). Without doubt is the liver the major site of Lp(a) clearance followed to a much lesser extent by the kidney and the arterial wall (Cain et al. 2005; Hrzenjak et al. 2003). Renal arterio-venous differences in Lp(a) concentrations suggest that the kidney can extract substantial amounts of Lp(a) from the circulation (Kronenberg et al. 1997). A metabolic role for the kidney is further supported by the inverse correlation between plasma Lp(a) and glomerular filtration rate (GFR), with a significant increase in Lp(a) in patients with more advanced chronic kidney disease (CKD) (Kronenberg 2014a) and by in vivo kinetic studies demonstrating diminished clearance of Lp(a) in CKD patients treated with hemodialysis (Frischmann et al. 2007). Further support for a possibly direct catabolic function of the kidney for Lp(a) came from the discovery of fragments of apo(a) in human urine and the decreased urinary excretion of apo(a) in patients with renal dysfunction (Kostner et al. 1996; Mooser et al. 1996).

Immune-Histochemical Studies

Previous immune-histochemical studies (Nakahara et al. 1999; Sato et al. 1993; Suzuki et al. 1997; Takemura et al. 1993) demonstrated apo(a) and apoB staining in human kidney biopsies from patients with various kidney diseases. No Lp(a) could be detected on the other hand in normal kidney tissue. In these studies, the authors therefore concluded a role of Lp(a) in the progression of renal disease rather than a direct function of the kidney for catabolizing Lp(a). Unfortunately, individual Lp(a) plasma concentrations were not provided in these studies. The negative immunostaining results could thus have resulted from the chance selection of probands with no or very low Lp(a) concentrations.

We therefore performed immune-histochemical studies of Lp(a) on normal human kidney tissue in relation to Lp(a) concentration and apo(a) size polymorphism in order to better understand the role of the kidney in Lp(a) metabolism (Haiman et al. unpublished).

Apo(a) was localized in glomeruli, capillaries, and blood vessels. Staining was found in glomerular capillaries and mesangial cells, but neither in Bowman's capsule nor in podocytes. As shown in Fig. 12.1a–f, the staining intensity strongly depended on the Lp(a) plasma concentration. Apo(a) staining in mesangial cells appeared as granular pattern and could be seen in main cell bodies, as well as in the faint processes of mesangial cells (Fig. 12.2). Staining of apo(a) in walls of capillaries and blood vessels between tubuli was variable: In most samples, apo(a) immunoreactivity was present in almost each capillary and blood vessel (Fig. 12.2a–d), but in some samples staining in these areas was seen very rarely. A positive staining was never observed in proximal and distal convolute tubuli (Fig. 12.3), as well as in collecting tubuli. The negative controls in the absence of primary antibodies did not show any staining (not shown). Staining of a tissue sample from a patient with very low plasma Lp(a) concentration which served as an additional negative control is shown in Fig. 12.1a.

ApoB immunostaining was localized in glomeruli, capillaries, blood vessels and faintly on erythrocyte membranes (Fig. 12.3). In glomeruli, apoB was found in glomerular capillaries and in mesangial cells, but never in Bowman's capsule nor in podocytes. The intensity of apoB-staining did not depend on the Lp(a) concentration in the plasma of the patients (not shown). Apart from a granular pattern, apoB staining was also seen diffuse in the cytoplasm of mesangial cells. It could also be

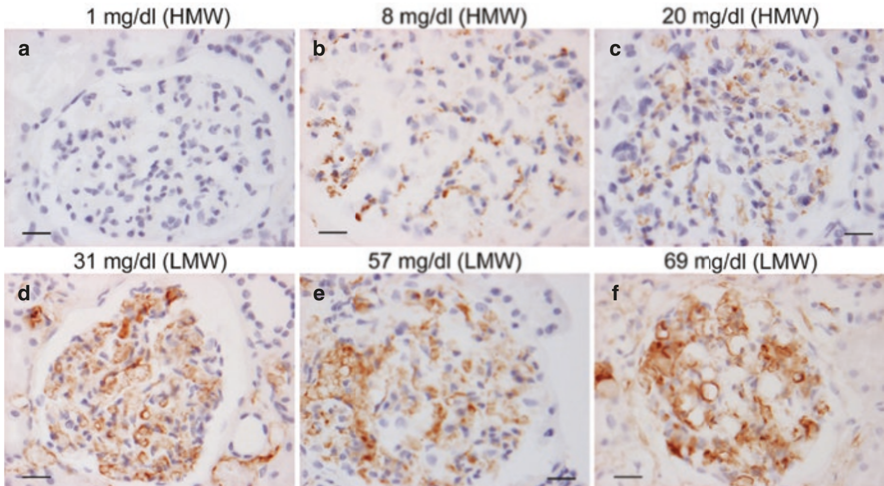

Fig. 12.1 Immunoreactivity of apo(a) in glomeruli of different tissue sections. Lp(a) plasma concentrations and apo(a) isoforms are indicated. The intensity of apo(a) staining in the glomeruli depends on Lp(a) concentrations (**a**–**f**). HMW, high molecular weight apo(a) phenotype; LMW, low molecular weight apo(a) phenotype; bars 20 μm

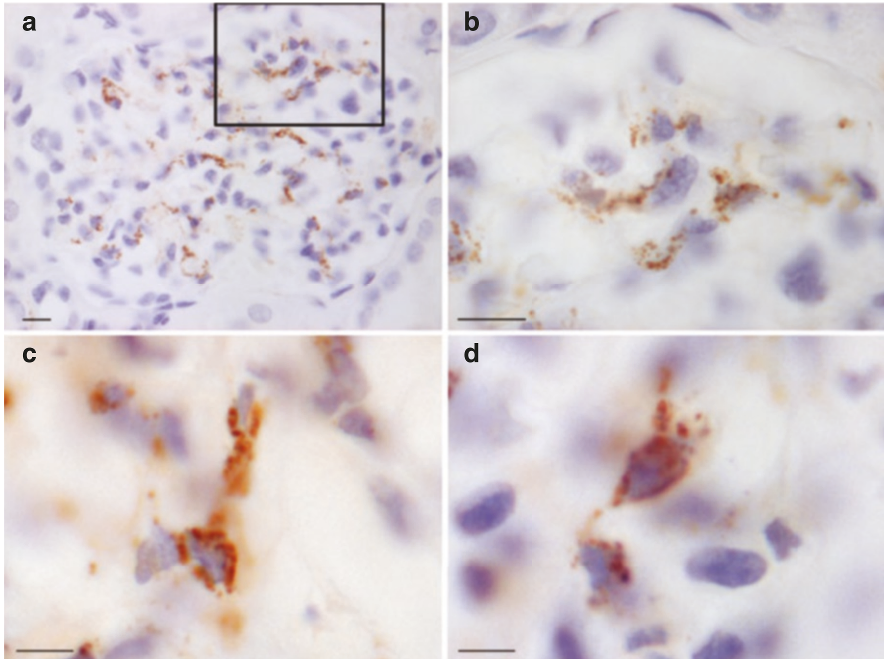

Fig. 12.2 Immunoreactivity of apo(a) in the glomerulum shows apo(a) in mesangial cells (**a**). Higher magnification of rectangle from picture **a** (**b**). Granular pattern of apo(a) staining in main cell body and processes of mesangial cells, (**c**, **d**) Mesangial cells with typical staining. Bars 10 μm

found on the walls of the capillaries between tubuli and the blood vessels (Fig. 12.3e–g) and on the membranes of erythrocytes. Similar to apo(a), no positive staining was ever observed for apoB in proximal and distal convolute tubuli (Fig. 12.3h), as well as in collecting tubuli. The negative controls did not show any staining (not shown), but staining of a tissue sample from a patient with very low Lp(a) plasma concentration demonstrated apoB immunoreactivity (not shown).

Apo(a) and apoB colocalized in mesangial cells (Fig. 12.4), capillaries, and blood vessels (data not shown). The negative controls did not show any non-specific staining (not shown).

Mesangial cells contribute to the regulation of glomerular filtration, produce mesangial matrix, and are continuously exposed to the plasma compartment, only separated from the capillary lumen by a fenestrated endothelium without intervening basement membrane (Latta 1992). Mesangial cells participate in a number of glomerular diseases and it is still a matter of debate whether lipids/lipoproteins selectively enhance mesangial matrix synthesis, proliferation of human mesangial cells, and foam cell formation—that may induce renal damage or diseases caused by lipid/lipoprotein deposition—induce injuries of mesangial cells (Gyebi et al. 2012; Mondorf et al. 1999; Wheeler et al. 1994). It has been postulated that the interaction between plasma lipoproteins and mesangial cells plays a major role in

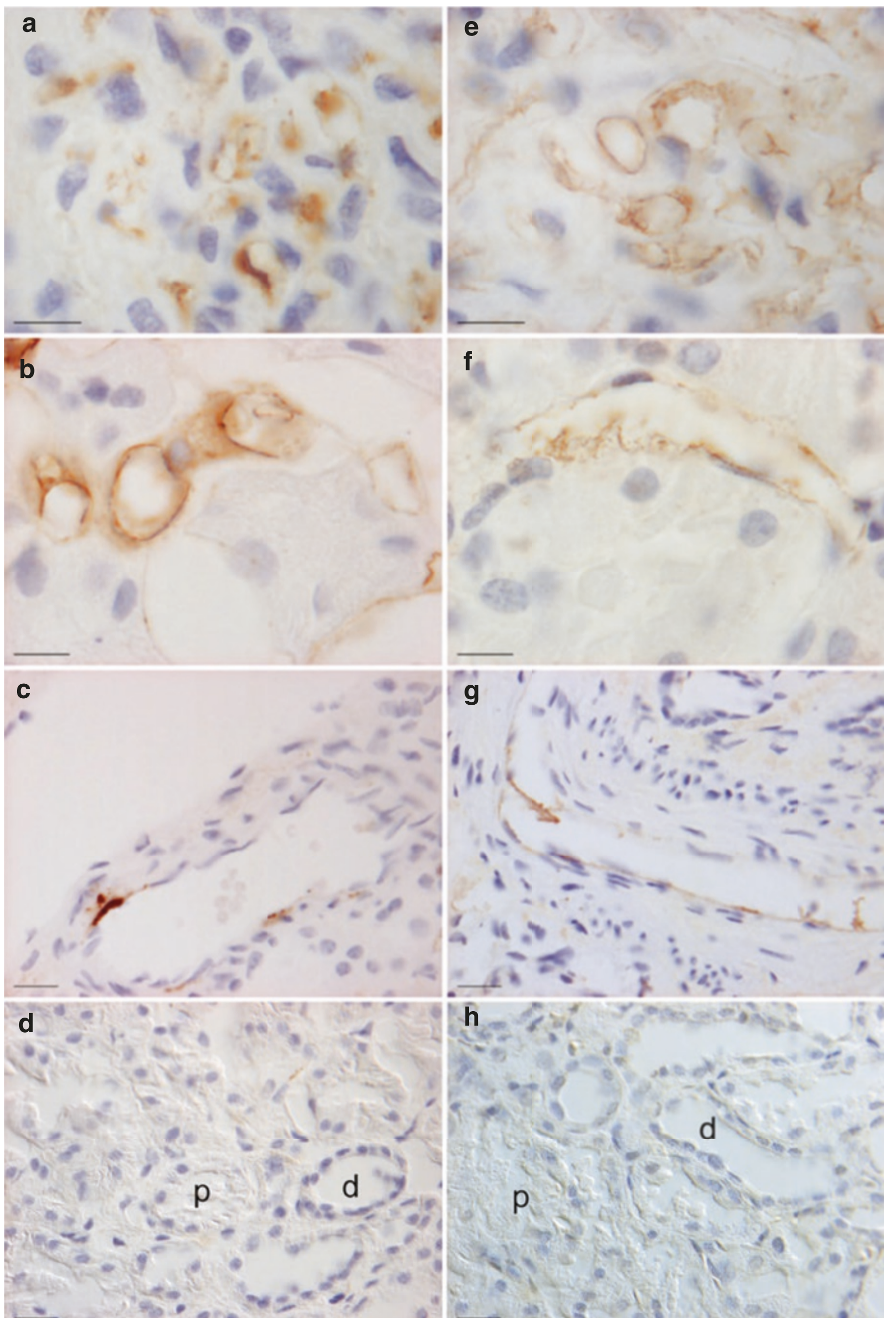

Fig. 12.3 Immuno-peroxidase staining of apo(a) (**a**–**d**) and apoB (**e**–**h**) in different regions of the human kidney tissue: (**a**, **e**) capillaries in glomeruli, (**b**, **f**) capillaries between tubuli, (**c**, **g**) blood vessel, (**d**, **h**) proximal (p) and distal (d) convoluted tubuli (DIC image). Bars: (**a**, **b**, **e**, **f**) 10 μm; (**c**, **d**, **g**, **h**) 20 μm

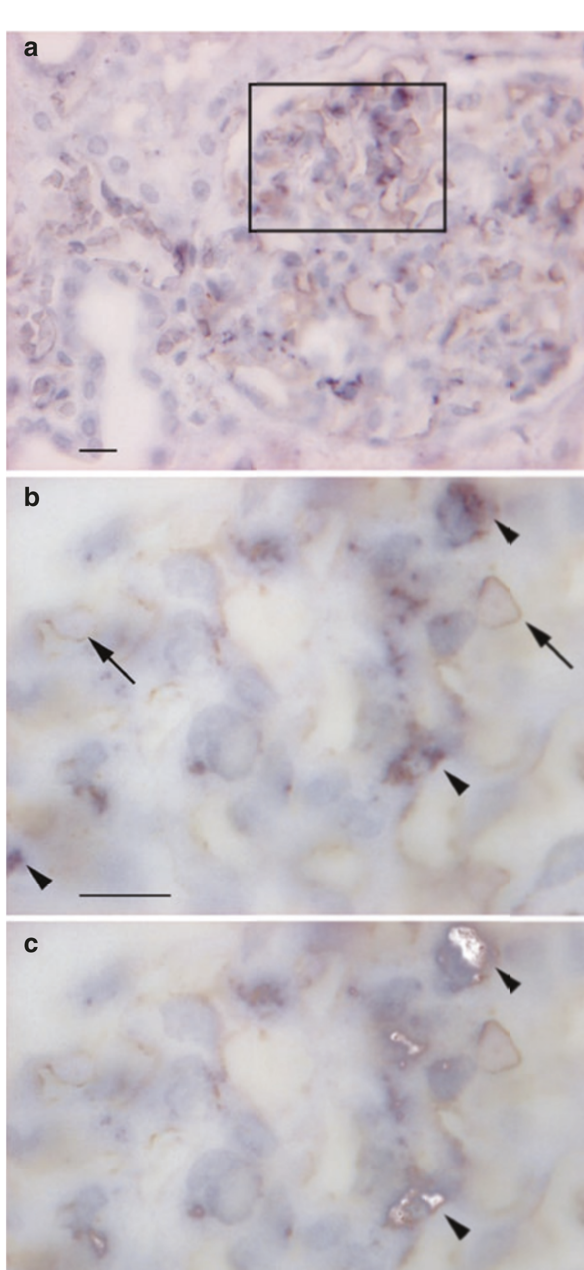

Fig. 12.4 Double staining of apo(a) and apoB in a glomerulum (**a**). Apo(a) is stained dark purple and apoB brown. Higher magnification of rectangle from picture **a** (**b**). Arrows show capillaries and arrowheads mesangial cells. (**c**) Digital image processing of picture **b**. The original dark purple color for apo(a) was replaced with white to demonstrate the sites of colocalization in the tissue (Adobe Photoshop). Arrowheads show mesangial cells. Bars: (**a**) 20 μm, (**b**, **c**) 10 μm

the pathophysiology of glomerulosclerosis. Several studies have shown that, in addition to native LDL, mesangial cells bind VLDL (Kramer-Guth et al. 1996a), oxidized LDL (Kramer-Guth et al. 1996a; Greiber et al. 1996), native and oxidized Lp(a) (Kramer-Guth et al. 1996b) in vivo and in vitro, in line with our immunohistochemical findings, and supporting the hypothesis that lipoproteins may play a critical role in mediating the development of glomerulosclerosis (Gröne et al. 1992).

To reveal the origin of immunologically detected apo(a) and apoB in mesangial cells, we investigated whether mRNA for apo(a) and apoB is expressed in human kidney. RT-PCR was performed with purified total RNA from three different kidney tissue samples, obtained from patients with different plasma Lp(a) concentrations, primary mesangial and proximal tubule cells, as well as from apo(a)-transfected HepG2 cells and human liver tissue. In these studies, apo(a) was only present in apo(a)-transfected HepG2 cells and human liver tissue. ApoB expression in human kidney tissue and in primary mesangial cells was very small compared to HepG2 and human liver tissue (data not shown).

Our findings of apo(a) and apoB co-localization in healthy human kidney tissue are in accordance with the previously observed intracellular accumulation of Lp(a) in rat kidneys after intravenous injection of human Lp(a) (Reblin et al. 2001) suggesting renal uptake and/or degradation of Lp(a) as a holoparticle. They are also in line with in vivo kinetic studies in hemodialysis patients resulting in diminished clearance of Lp(a) in these patients (see section "In Vivo Studies" (Frischmann et al. 2007)).

Role of LDL Receptor and Other Receptors for Lp(a) Clearance

Due to the structural similarities between LDL and Lp(a), the LDL receptor (LDLR) has been investigated and discussed extensively as a candidate receptor for Lp(a) over the past decades. Strong arguments again a role of the LDLR for Lp(a) uptake and degradation came from numerous reports that statins which enhance LDLR expression and thereby markedly reduce LDL, have a neutral, or an even elevating effect on plasma Lp(a) concentrations (Khera et al. 2014; Tsimikas et al. 2020; Willeit et al. 2018). Unexpectedly, PCSK9 inhibitors, which also increase the cell surface expression of the LDLR, via an inhibition of LDLR intracellular degradation, not only lower LDL-C by 50–60%, but also reduce plasma Lp(a) concentrations by 20–30% (McKenney et al. 2012). This observation has renewed research into the roles of PCSK9 and of the LDLR in mediating the clearance of Lp(a) from the circulation.

Several earlier reports from in vitro studies suggested, however, a role of the LDLR for Lp(a) uptake, as well. Initial reports showed that Lp(a) can bind to the LDLR, albeit with a lower affinity than LDL (Armstrong et al. 1990; Havekes et al. 1981; Reblin et al. 1997; Snyder et al. 1992). Lp(a) was also proposed to associate with LDL and undergo LDLR-mediated clearance via a "hitch-hiking" mechanism (Hofer et al. 1997; Kostner 1993). In HepG2 and primary human fibroblasts, PCSK9 was shown to reduce the binding and the cellular uptake of Lp(a) via the LDLR

(Raal et al. 2014). In contrast, other studies found no significant role for the LDLR in mediating the cellular uptake of Lp(a) in primary human hepatocytes and HepG2 cells (Sharma et al. 2017; Villard et al. 2016). Moreover, no significant difference was found in the cellular uptake of Lp(a) in primary lymphocytes derived from normolipemic individuals compared with patients with homozygous familial hypercholesterolemia (FH) and complete absence of LDLR function (Chemello et al. 2020).

Multiple alternative pathways for Lp(a) clearance using other receptors have been proposed, as elegantly reviewed by McCormick and Schneider (2019). For instance, in macrophages, the Toll-like receptor 2 (TLR2) acts as a receptor for Lp(a)-bound oxidized phospholipids (oxPL). This observation is in line with a large, although non-significant, genome-wide association study (GWAS) showing that TLR2 is the only receptor associated with circulating Lp(a) concentrations (Mack et al. 2017; Seimon et al. 2010). Likewise, the scavenger receptor BI (SR-BI) has been shown to promote the selective uptake of Lp(a) cholesterol esters in cells and in SR-BI transgenic and knockout mice (Yang et al. 2013).

Due to the high degree of glycosylation of apo(a), carbohydrate-binding proteins (lectins), such as the asialoglycoprotein receptor (ASGPR), have also been shown to act as Lp(a) receptors in mice (Hrzenjak et al. 2003), but not all findings are consistent (Cain et al. 2005; Sharma et al. 2017). Given the strong homology between apo(a) and plasminogen, the role of plasminogen receptors in mediating Lp(a) clearance has been investigated (Tam et al. 1996). One of such receptors, the plasminogen receptor presenting a C terminal lysine ($PLGR_{KT}$), was shown to mediate the cellular uptake of Lp(a) by human hepatoma cells and primary human fibroblasts. This study also showed that the LDL component of Lp(a) undergoes lysosomal degradation whereas apo(a) traffics through recycling endosomes and is re-secreted into the medium (Sharma et al. 2017). However, the concentration of free apo(a) in human plasma is relatively low, which suggests minimal to no recycling of apo(a) in the circulation.

Several other members of the LDLR family of receptors have also been proposed to mediate whole Lp(a) particle cellular uptake. Thus, the VLDL receptor binds apo(a) and allows the internalization and subsequent degradation of Lp(a) in macrophages (Argraves et al. 1997). The LDLR-related protein 1 (LPR1) and megalin/gp330 (known as LRP2) also play a role in Lp(a) binding, cellular uptake, and degradation in vitro (Reblin et al. 1997; März et al. 1993; Niemeier et al. 1999). LRP8 is also able to bind Lp(a) at the plasma membrane, but it remains to be shown whether this promotes cellular uptake and degradation of Lp(a) particles (Steyrer and Kostner 1990). The cellular uptake of Lp(a) in HepG2 hepatoma cells was, however, recently shown to be unaffected overexpressing either the VLDLR, LRP1, or LRP8 (Romagnuolo et al. 2017).

Interestingly, GWAS studies could not identify a significant association between any of the proposed receptors and Lp(a) concentrations except the LDL receptor. This might be explained by the fact that Lp(a) also contains cholesterol and the signal with the LDL receptor might stem from the cholesterol content of Lp(a) (Mack et al. 2017; Hoekstra et al. 2021).

In Vivo Studies

In vivo kinetic studies using stable isotopes have been performed to investigate possible mechanisms underlying elevated Lp(a) concentrations in two different groups of kidney disease (Fig. 12.5). These studies also revealed insights into a possible role of the kidney in Lp(a) metabolism.

Patients with nephrotic syndrome (NS) revealed increased synthesis rates of Lp(a) without changes in the fractional catabolic rate indicating an increased production of Lp(a)—along with many other proteins—rather than a decreased catabolism (de Sain-van der Velden et al. 1998). These results have to be, however, taken cautiously since they are based on kinetic data from only five patients and five controls with widely varying single values. Therefore, although mean values between patients and controls look impressively different (see Fig. 12.5), production rates do not differ significantly between these two groups after statistical evaluation of the provided single data. Therefore, human kinetic studies should be repeated with higher numbers of included patients/controls to confirm the previously published analysis. It has been demonstrated that in NS, patients lose a significant amount of proteins via urine, and that the increased synthesis of Lp(a) might be a result of compensation to keep up the oncotic pressure in the circulating blood.

In contrast, CKD patients treated by hemodialysis (HD, essentially lacking renal function) showed similar synthesis rates for both apo(a) and apoB from Lp(a) between hemodialysis and healthy controls (Fig. 12.5). The fractional catabolic rates (FCR) for both components of Lp(a), however, were significantly lower in HD patients compared with controls. This resulted in a much longer residence time of 8.9 days for Lp(a)-apo(a) and 12.9 days for Lp(a)-apoB in HD patients compared to controls (4.4

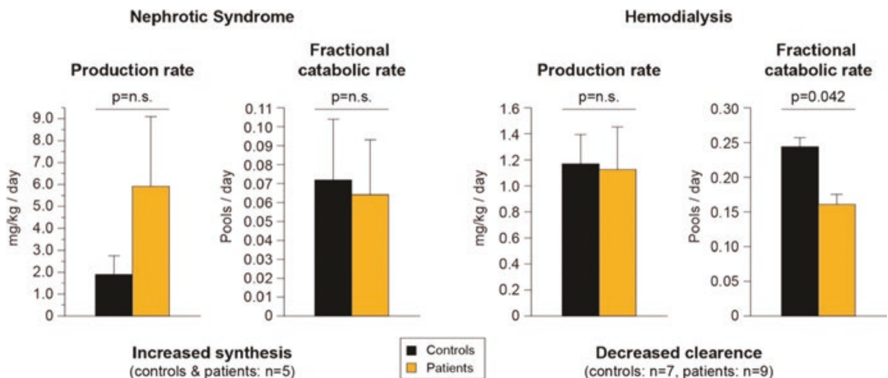

Fig. 12.5 In vivo kinetic studies using stable isotopes reveal different mechanisms leading to increased Lp(a) concentrations in patients with nephrotic syndrome (NS) and chronic kidney disease (CKD) treated with hemodialysis (HD): whereas in NS patients Lp(a) production rates are increased with no changes in catabolic rates, the situation in HD patients is opposite: Lp(a) concentrations are increased due to diminished catabolic rates and not synthesis. For NS patients, kinetic parameters are given as Lp(a) total protein, for HD patients as Lp(a)-apo(a). Each bar represents mean ± standard error. Data for NS patients are taken from de Sain-van der Velden et al. (1998), those for HD patients from Frischmann et al. (2007)

and 3.9 days, respectively) (Frischmann et al. 2007). These results suggest, together with the discovery of apo(a) immunostaining in healthy human kidney tissue (see section "Immune-Histochemical Studies"), a possible catabolic function for Lp(a) of the human kidney. The prolonged residence time of Lp(a) in HD patients might substantially contribute to the high risk of atherosclerosis in these patients (see below).

Lp(a) in Kidney Disease

Parra et al. reported for the first time in 1987 elevated Lp(a) concentrations in hemodialysis patients (Parra et al. 1987). Since then, interest in the role of the kidney in the metabolism of Lp(a) has steadily increased as documented in the comprehensive review articles (Kronenberg et al. 1996; Kronenberg 2014a; Hopewell et al. 2018). The earliest report related to this topic came from Papadopoulos et al., who described a higher frequency of a second pre-beta band in agarose gel electrophoresis in hemodialysis patients than in controls (Papadopoulos et al. 1980). Numerous studies have since then been published related to Lp(a) in nephrotic syndrome, CKD, or kidney transplantation (see a recent review by Enkhmaa and Berglund (2022)).

Proteinuria and Nephrotic Syndrome

Several studies reported elevated Lp(a) concentrations in patients with proteinuria or nephrotic syndrome (NS) (Brown et al. 1995; Faucher et al. 1993; Joven et al. 1995; Karádi et al. 1989; Querfeld et al. 1993; Stenvinkel et al. 1993; Takegoshi et al. 1991; Thomas et al. 1992; Wanner et al. 1993; Hong and Yang 1995; Vaziri 2016). In contrast to hemodialyzed CKD patients, pronounced increases in Lp(a) concentrations were reported in all apo(a) isoform groups (Wanner et al. 1993). In the largest study, Kronenberg et al. reported five-fold elevated Lp(a) plasma concentrations in non-diabetic NS patients compared to controls (Kronenberg et al. 2004). While the increase was partly explained by different distribution of apo(a) isoforms between NS patients and controls, both small and large isoform groups were significantly associated with higher Lp(a) concentrations in NS patients compared to controls. Elevated Lp(a) levels in NS have been demonstrated in longitudinal studies to decrease after remission of nephrotic syndrome (Faucher et al. 1993; Joven et al. 1995; Stenvinkel et al. 1993; Takegoshi et al. 1991; Wanner et al. 1993).

Early Stages of Kidney Disease

Increased Lp(a) concentrations have been observed in patients with reduced kidney function characterized by impaired glomerular filtration rates (GFR) (Catena et al. 2015; Kovesdy et al. 2002; Kronenberg et al. 2000; Lin et al. 2014; Milionis et al.

1999; Sechi et al. 1998). In only three of these studies, apo(a) isoforms were analyzed in addition to plasma Lp(a) concentrations (Kronenberg et al. 2000; Milionis et al. 1999; Sechi et al. 1998). Kronenberg et al. examined the association between kidney function, Lp(a) plasma concentrations, and apo(a) isoform size in multicenter design in 227 non-nephrotic patients with different degrees of kidney impairment. Lp(a) concentrations were significantly higher in patients with kidney disease compared with 227 age-, sex- and apo(a)-isoform-matched controls (Kronenberg et al. 2000). Lp(a) were increased already in the earliest stages of kidney impairment before GFR starts to decrease. Kidney function was inversely related with Lp(a) concentrations, independent of the initial kidney disease. Most remarkably, the inverse association between Lp(a) values and kidney function was only seen in the subgroup of patients with HMW apo(a) isoforms, in line with observations in hemodialyzed CKD patients (see section "Chronic Kidney Disease Treated by Hemodialysis or Peritoneal Dialysis" (Dieplinger et al. 1993; Kronenberg et al. 1995)).

Inverse correlations between Lp(a) concentrations and GFR were also found in the Penn Diabetes Heart Study based on 1.852 patients with mild kidney impairment (Lin et al. 2014) and in a population study involving 7.675 individuals from different ethnic backgrounds, particularly in non-Hispanic blacks, eventually suggesting ethnic differences (Kovesdy et al. 2002).

However, the observed association between Lp(a) and GFR in the above-mentioned studies could not be confirmed by others: there was no significant association described in 804 individuals with stage 3–4 CKD and also no suggestion of an interaction with apo(a) isoform size (Uhlig et al. 2005). Furthermore, a study of 87 kidney donors whose average kidney function was reduced from a GFR of 112 before donation to 72 mL/min/1.73 m^2 one year later showed no significant difference in Lp(a) plasma values as a result of donation (Doucet et al. 2016). The reason for the described discrepancies remains unclear; an explanation for the findings in kidney transplant patients may be caused by an influence of immunosuppressive medications on Lp(a) concentrations.

Chronic Kidney Disease Treated by Hemodialysis or Peritoneal Dialysis

The majority of studies of Lp(a) in CKD is devoted to hemodialysis patients and reported significantly elevated Lp(a) plasma concentrations in these patients (Parra et al. 1987; Dieplinger et al. 1993; Kronenberg et al. 1995; Auguet et al. 1993; Barbagallo et al. 1992, 1993; Cressman et al. 1992; Fiorini et al. 1995; Gault et al. 1995; Haffner et al. 1992; Heimann et al. 1991; Hirata et al. 1993; Kandoussi et al. 1992; Okura et al. 1993; Parsi et al. 1988; Shoji et al. 1992; Webb et al. 1993; Gambhir et al. 2013; Parsons et al. 2003). Few studies described Lp(a) values not significantly different from those in controls (Buggy et al. 1993; Docci et al. 1994; Irish et al. 1992). Similar observations of elevated Lp(a) concentrations were made in patients treated by continuous ambulatory peritoneal dialysis (CAPD) (Querfeld

et al. 1993; Kronenberg et al. 1995; Barbagallo et al. 1993; Gault et al. 1995; Shoji et al. 1992; Webb et al. 1993; Buggy et al. 1993; Irish et al. 1992; Anwar et al. 1993; Murphy et al. 1992; Thillet et al. 1994; Wanner et al. 1995). Only one study reported lower Lp(a) values in CAPD patients (Kandoussi et al. 1992). In these studies, the range of differences between controls and patients was extremely broad. These inconsistent findings can be explained by the low number of patients and controls in several studies together with an up to 1.000-fold inter-individual variation in Lp(a) concentrations and the otherwise strong genetic control of Lp(a) concentrations. These circumstances require large numbers of investigated individuals to reveal reliable results as discussed earlier (Kronenberg et al. 1996; Kronenberg 2014b).

Only few studies with an adequate number of patients have determined apo(a) isoforms in addition to Lp(a) concentrations (Dieplinger et al. 1993; Auguet et al. 1993; Hirata et al. 1993; Wanner et al. 1995).

To overcome the limitations of small sample size, Kronenberg et al. performed a large multicenter study that included 534 hemodialysis and 168 CAPD patients (Kronenberg et al. 1995). Both patient groups showed significantly elevated Lp(a) levels in comparison with the controls. Lp(a) values were significantly higher in patients treated with CAPD than with hemodialysis. Notably, the elevations on Lp(a) in hemodialysis and CAPD patients were less pronounced than in several other small studies. Consideration of apo(a) phenotypes revealed that the increased concentration of Lp(a) was not explained by different frequencies of apo(a) isoforms between patients and controls confirming and extending earlier findings (Dieplinger et al. 1993). Therefore, elevated Lp(a) values in CKD are caused by the disease and are not due a higher frequency of LMW apo(a) phenotypes in patients. The reason for the selective elevation of Lp(a) levels in HMW isoforms in both treatment groups is presently unclear.

Similar to nephrotic syndrome, the markedly elevated Lp(a) concentrations in CAPD patients are probably caused by the high loss of protein, in this case through the dialysate fluid as demonstrated by Kronenberg et al. (1995). A generally increased hepatic synthesis and secretion of lipoproteins including Lp(a) is the most likely reason for their higher Lp(a) values. This increased synthesis of Lp(a) might be responsible for the trend to higher Lp(a) values in CAPD patients with LMW apo(a) isoforms, which, however, did not reach statistical significance.

Once the final CKD stage is reached, the cause of kidney disease has no influence on Lp(a) plasma concentrations (Kronenberg et al. 1995). This observation was confirmed by a study including hemodialysis, CAPD, and renal transplant patients by reporting similar Lp(a) values in patients with and without insulin-dependent diabetes mellitus (Gault et al. 1995).

The mechanism underlying Lp(a) elevation in CKD is still not fully understood. The rapid decrease of Lp(a) following renal transplantation, as outlined in section "Kidney Transplantation", argues against an elevation induced by an acute phase reaction, as was suggested earlier (Levine and Gordon 1995). Human kinetic studies in various groups of these patients have been performed to examine whether this elevation is caused by synthesis or catabolism. Further mainly kinetic studies are necessary to shed light on the apo(a)-isoform-specific elevation of Lp(a) and its possible clinical impact.

Kidney Transplantation

Findings of elevated Lp(a) concentrations in various patient groups with CKD led several researchers to study the influence of kidney transplantation on Lp(a) plasma concentrations. Black and Wilcken were the first to observe a highly significant decrease in Lp(a) in 20 patients following renal transplantation (Black and Wilcken 1992). The results from several subsequent studies were not consistent, probably reflecting differences in the study design. All prospective longitudinal studies clearly showed a decrease in Lp(a) following transplantation (Gault et al. 1995; Azrolan et al. 1994; Kronenberg et al. 1993, 1994a; Murphy and McNamee 1992; Murphy et al. 1995; Segarra et al. 1995; Yang et al. 1994). Lp(a) changes were independent of the modality of immunosuppressive therapy.

Lp(a) decreased after kidney transplantation in CKD patients, previously treated by CAPD, independently of their apo(a) isoform. In contrast, in previously hemodialyzed patients, Lp(a) declined after kidney transplantation only in those with large apo(a) isoforms (Enkhmaa et al. 2016; Kerschdorfer et al. 1999; Kronenberg et al. 1994a, 2003; Rosas et al. 2008). These findings are in line with the previously described increased Lp(a) concentrations depending on the apo(a) size.

These results, together with those of kinetic studies in hemodialysis patients (Frischmann et al. 2007), are a further convincing indication of a metabolic role of the kidney in Lp(a) catabolism and that the observed Lp(a) changes are due to loss of functional kidney tissue.

Lipoprotein(a) and Cardiovascular Outcome in CKD

Cardiovascular disease is also a major cause of death in CKD patients (Chronic Kidney Disease Prognosis Consortium et al. 2010). At the same time, CKD is considered as one of the “Big Five” contributing to cardiovascular disease (Kronenberg and Schernthaner 2020). The numerous reports on Lp(a) as risk factor for atherosclerosis and cardiovascular disease in the general population have therefore encouraged researchers to investigate the predictive power of Lp(a) also in CKD. Early studies by Cressman et al. found significantly higher L(a) concentrations in hemodialysis patients with events than in those without events (78.9 mg/dL vs 35.4 mg/dL; $P < 0.001$) (Cressman et al. 1994). Various inconsistent subsequent studies, many with limited statistical power or using various control groups or reference Lp(a) values, followed and examined whether Lp(a) concentrations contribute to increased cardiovascular risk in CKD patients (Bajaj et al. 2017; Emanuele et al. 2004; Koch et al. 1997; Kronenberg et al. 1999; Longenecker et al. 2005; Ohashi et al. 1999; Shlipak et al. 2005; Webb et al. 1995).

In the Cardiovascular Health Study in elderly individuals, there was no significant association between Lp(a) and cardiovascular mortality in CKD patients observed (Shlipak et al. 2005). However, the risk estimate was comparable to that observed in those individuals without CKD in whom a significant association between Lp(a) and cardiovascular mortality was reported.

In line with the above-mentioned apo(a) isoform-specific elevations of Lp(a) in non-nephrotic CKD, the majority of cross-sectional as well as prospective studies revealed that Lp(a) concentrations and, even more, the LMW apo(a) isoform are independent risk factors for cardiovascular disease in CKD (Cressman et al. 1992; Koch et al. 1997; Kronenberg et al. 1994b, 1999; Longenecker et al. 2002, 2005).

Similar observations have been made by Wanner et al., who investigated CHD in 62 CAPD patients (Wanner et al. 1995). Affected patients showed only a small elevation of Lp(a) (51 mg/dL vs 39 mg/dL; $P = 0.06$), but a significantly higher frequency of LMW apo(a) phenotypes compared with patients without CHD (67% v 31%; $P < 0.05$).

Kollerits et al. reported an association of elevated Lp(a) concentrations and LMW apo(a) isoforms with an increased risk for all-cause mortality and death due to infection in hemodialysis patients with type 2 diabetes mellitus in the 4D Study (Kollerits et al. 2016). Their findings were modified by age; the association between apo(a) isoforms and mortality was only seen in patients ≤66 years.

CKD patients with LMW apo(a) isoforms have generally higher Lp(a) plasma concentrations during their entire life, whereas patients with HMW isoforms develop high Lp(a) concentrations only when renal insufficiency begins to develop. CKD is therefore the only constellation in which the apo(a) isoform has a higher predictive power for atherosclerosis than the Lp(a) concentration. This might explain why the apo(a) isoform, which reflects pre-disease Lp(a) values, is an excellent predictor of atherosclerosis in CKD patients before a clinical atherosclerotic complication develops (Kronenberg et al. 1996; Kronenberg 1995).

In addition to these genetic considerations, it has been postulated that prolonged residence times of Lp(a), as shown in hemodialyzed patients by in vivo kinetic studies (Frischmann et al. 2007), may contribute to the high risk of atherosclerosis in CKD patients.

Metabolic Relation Between Kidney and Lipoprotein(a): Conclusions and Gaps in Knowledge

In contrast to the general population, the elevated Lp(a) plasma concentrations in CKD patients are nongenetic in origin and are a consequence of the disease. Numerous studies reporting increased Lp(a) values in CKD suggested a role of the kidney in the catabolism of Lp(a). However, only few studies, such as human kinetic studies (Frischmann et al. 2007), provided at least indirect evidence to support this hypothesis.

There are two possible explanations for high Lp(a) concentrations in CKD. First, the kidney has an indirect influence on the synthesis of Lp(a) in the liver. This might be triggered by a factor that is secreted by the kidney and regulates hepatic Lp(a) synthesis. This idea is supported by the findings of Azrolan et al. in five nephrectomized hemodialysis patients who had Lp(a) concentrations that were not different from those in a control group (Azrolan et al. 1994). The authors therefore proposed that impaired or dysfunctional kidneys might play a role in elevating plasma Lp(a)

concentrations. These results remain to be confirmed in longitudinal studies and considering apo(a) isoforms.

The second explanation for high Lp(a) concentrations in CKD is that the kidney has a direct catabolic function and degrades Lp(a). Oida et al. were the first to describe the excretion of degraded lipid-free apo(a) fragments in urine, which decreased with the decline in glomerular filtration rate (Oida et al. 1992). Several subsequent studies were published confirming the generation of apo(a) fragments (Kostner et al. 1996; Cauza et al. 2003; Trenkwalder et al. 1997). A direct role of the human kidney in catabolizing Lp(a) was further supported by reporting clear differences in Lp(a) plasma concentrations between arteria and vena renalis in humans (Kronenberg et al. 1997). Various renal cell types express the LDL receptor-related protein (megalin), a member of the LDL receptor gene family (Kukida et al. 2020), which is believed to play a role in the catabolism of Lp(a) (März et al. 1993) (see also section "Clearance and Catabolism").

On the other hand, Lp(a) also could play a role in the pathogenesis of kidney disease. If elevated Lp(a) concentrations are a primary cause of kidney disease, one would expect a higher frequency of LMW apo(a) isoforms in CKD patients. However, this was not observed in two large studies (Dieplinger et al. 1993; Kronenberg et al. 1995). It is conceivable, however, that high Lp(a) values accelerate the progression of renal disease at a later stage. Lipoprotein(a) and apoB have been demonstrated in the glomeruli of patients with glomerular disease, mainly in the mesangial area and occasionally along capillary loops (Sato et al. 1993).

At least one important question regarding the metabolic interrelationship between Lp(a) and the kidney remains unanswered: Why are Lp(a) concentrations in hemodialyzed CKD patients elevated only in carriers of HMW apo(a) isoforms? Kinetic human studies performed in larger study groups (including a sufficiently large subgroup with HMW apo(a) isoforms) should help to clarify this issue.

Acknowledgments Work from the author described in this overview has been supported by grants from the Austrian Science Fund (P12358-MED) and the Jubiläumsfonds of the Austrian National Bank (6721/4).

The author highly appreciates the critical reading of this review manuscript by Florian Kronenberg as well as fruitful and long-lasting collaborations with many scientific colleagues and technical assistants including H Andersson, M Auinger, D Bach, U Beisiegel, C Bösmüller, JH Bräsen, B Dieplinger, N Donarski, E Dosch, C Drechsler, A von Eckardstein, G Friedrich, ME Frischmann, B Grabensee, H Graf, E Gröchenig, A Gruber, M Hohenegger, F Hoppichler, E Hoye, K Huber, K Ikewaki, H Kathrein, L Kerschdorfer, B Kollerits, P König, GM Kostner, KM Kostner, V Krane, F Kronenberg, MF Kronenberg, C Lackner, C Lamina, U Lang, I Leiter, K Lhotta, A Lingenhel, EM Lobentanz, R Margreiter, W März, G Maurer, HJ Menzel, N Moes, U Neyer, D Öfner, G Pinter, A Pribasnig, B Rantner, T Reblin, J Reitinger, P Riegler, E Ritz, H Salmhofer, W Salvenmoser, C Sandholzer, JR Schäfer, K Scheiber, U Scheidle, M Schober, JP Schwaiger, H Schweer, RA Stahl, T Stefenelli, W Sturm, F Thaiss, J Thiery, E Trenkwalder, G Utermann, C Wanner, M Wieshofer, G Wolf.

My special thanks go to Linda Fineder for a tremendous 30-year-long collaboration.

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Chapter 13
Lp(a) as a Cardiovascular Risk Factor

Angela Pirillo and Alberico Luigi Catapano

Introduction

Epidemiological and genetic evidence has clearly shown that elevated levels of lipoprotein(a) [Lp(a)] are causally linked with an increased risk of cardiovascular disease (CVD). This has led to renewed interest in an "old" lipoprotein that, although sharing structural similarities with LDL, is endowed with exclusive properties due to the presence of apolipoprotein(a) [apo(a)], a protein with homology to plasminogen (McLean et al. 1987). Lp(a) exerts multiple effects in CVD, as it can act similarly to an LDL particle and enter the intima of the arterial wall, thus contributing to atherosclerosis, but it can also inhibit fibrinolysis due to its homology with plasminogen and contribute to inflammation by mean of Lp(a)-associated oxidized phospholipids (Koutsogianni et al. 2021).

Apo(a), encoded by the *LPA* gene, is a highly heterogeneous protein containing multiple repeats of kringle 4 type 2 (KIV2). The number of repeats is genetically determined by common copy-number variation within the *LPA* gene and is inversely related to the plasma concentration of Lp(a), with isoforms containing fewer KIV2 repeats being associated with smaller Lp(a) lipoprotein size and higher circulating levels. Two unique features of Lp(a) are its wide range of plasma level variation

A. Pirillo
Center for the Study of Atherosclerosis, E. Bassini Hospital, Milan, Italy

IRCCS MultiMedica, Milan, Italy
e-mail: angela.pirillo@guest.unimi.it

A. L. Catapano (✉)
IRCCS MultiMedica, Milan, Italy

Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Milan, Italy
e-mail: alberico.catapano@unimi.it

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_13

(from <0.2 to >200 mg/dL, or <0.5 to 500 nmol/L), which for the most part reflects genetic variations in *LPA*, and its profile of distribution in the population, which is highly skewed with a long tail toward extremely high values, with ~20% of individuals showing Lp(a) levels >50 mg/dL (Nordestgaard et al. 2010). Elevated Lp(a) levels can occur in individuals with otherwise normal lipid levels; the risk threshold for Lp(a) is set at 50 mg/dL.

Being mostly genetically determined, circulating levels of Lp(a) are relatively stable throughout life. Based on this observation, current European guidelines for the management of dyslipidemias recommend measuring Lp(a) at least once in life (Mach et al. 2020).

Lp(a) and CVD: A Causal Relationship

A large number of studies have established a causal relationship between Lp(a) and CVD (Emerging Risk Factors Collaboration et al. 2009); above all, elevated Lp(a) increases the risk of myocardial infarction (MI), stroke, and peripheral arterial disease, but its pathophysiological role appears to be more complex than that of LDL. In fact, the mechanisms beyond this association likely involve both its LDL particle-like features (promoting atherosclerosis) and plasminogen-like particle (inhibiting fibrinolysis).

A stepwise increase in the risk of MI with increasing levels of Lp(a) was reported in both genders in a general European population, with extreme levels of Lp(a) (>95th percentile) predicting a threefold to fourfold increased MI risk (Kamstrup et al. 2008). In agreement with this observation, another study reported a threefold to fourfold higher prevalence of ASCVD and MI in adults having Lp(a) >99th percentile [median Lp(a) 460 nmol/L] compared to those with Lp(a) levels ≤20th percentile [median Lp(a) 7 nmol/L] (Nurmohamed et al. 2021). The incorporation of Lp(a) into algorithms for CV risk assessment led to the increase of mean estimated 10-year risk and the upward reclassification of substantial percentages of patients, both in primary and in secondary prevention (Nurmohamed et al. 2021). An interesting observation reported in this study was a higher LDL-C goal attainment among individuals with lower Lp(a) levels compared with those with high Lp(a) levels, which may suggest that measured LDL-C in these patients mainly is the result of high Lp(a) levels (Nurmohamed et al. 2021). It has been observed that a 15 mg/dL (~0.39 nmol/L) increase in Lp(a) cholesterol determines a higher hazard ratio of CV mortality compared with a corresponding increase in LDL cholesterol (1.18 vs 1.05), which may suggest that not only the cholesterol content in lipoprotein(a) is pathogenic, but likely its unique protein apo(a) may play a relevant role as well (Langsted et al. 2019).

Mendelian randomization studies have substantiated the causal role of Lp(a) in CVD (Clarke et al. 2009; Kamstrup et al. 2009; Burgess et al. 2018; Lamina et al. 2019). Two common single-nucleotide polymorphisms (SNPs) have been strongly associated with both increased levels of Lp(a) and increased risk of coronary

disease (Clarke et al. 2009); on the other hand, a 10 mg/dL lower genetically determined Lp(a) level was associated with a 5.8% lower risk of coronary heart disease (CHD) (Burgess et al. 2018). The observation that a similar reduction in LDL-C will translate into a 14.5% lower CHD risk has a relevant consequence on the magnitude of Lp(a) level reduction required to provide a clinical benefit: a ~100 mg/dL Lp(a) reduction concentration anticipates a CHD risk reduction similar to that achieved with a ~39 mg/dL change in LDL-C level (Burgess et al. 2018) (Fig. 13.1). A similar finding has been reported by another study (Lamina et al. 2019) (Fig. 13.1).

Elevated Lp(a) levels can also explain, at least in part, the residual CV risk commonly observed in patients with well-controlled LDL-C levels. A meta-analysis of data from 29,069 patients included in seven placebo-controlled statin trials showed that elevated baseline and on-statin Lp(a) levels conferred a significantly higher CV risk, suggesting that statins do not impact the residual risk in patients with elevated Lp(a) (Willeit et al. 2018) (Fig. 13.2). Among patients with recent ACS receiving intensive or maximum-tolerated statin treatment, baseline Lp(a) levels predicted the risk of MACE, nonfatal MI, and CHD and CV death, independent of baseline LDL-C (Bittner et al. 2020). Although the reduction of MACE is mainly attributable to the reduction in LDL-C (referred to as "corrected LDL-C") across the range of baseline Lp(a) levels, in patients with the highest baseline Lp(a) levels the contribution of Lp(a) reduction in reducing the risk of MACE was substantial (Bittner et al. 2020). Interestingly, moderately elevated plasma Lp(a) levels (≥15 mg/dL) appear to confer an increased risk of all-cause mortality in patients with CAD (Liu et al. 2021).

The role of Lp(a) in determining the residual CV risk is further supported by the results of most recent trials evaluating therapies able to achieve considerable reductions in LDL-C levels. The FOURIER trial, which evaluated the clinical impact of

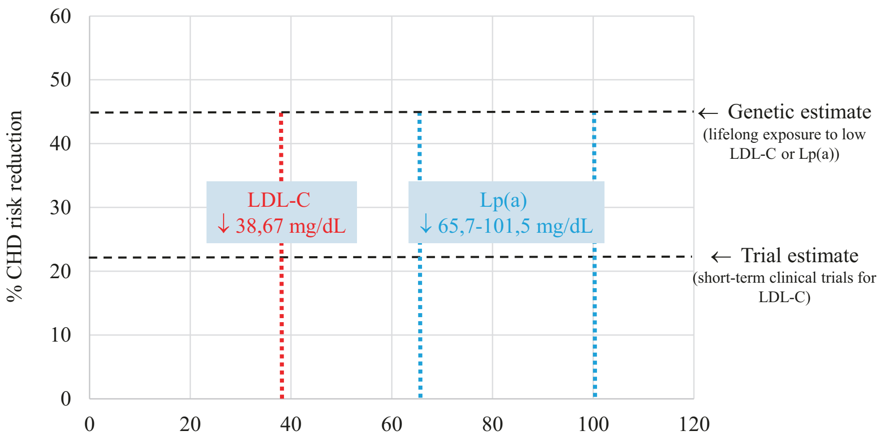

Fig. 13.1 Estimates of CHD risk reduction with lowering of LDL-C or Lp(a) level

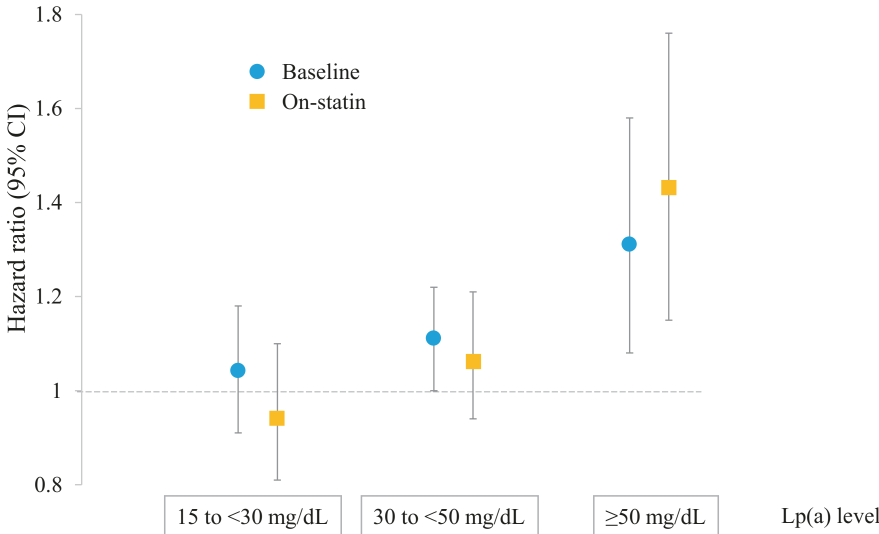

Fig. 13.2 Association of baseline and on-statin Lp(a) levels with incident CVD, age-adjusted, and sex-adjusted according to different Lp(a) levels

evolocumab in patients with ASCVD, found that higher baseline levels of Lp(a) were associated with an increased risk of coronary events, independent of LDL-C levels (O'Donoghue et al. 2019). Furthermore, a post hoc analysis of the ODYSSEY OUTCOMES trial observed that while patients having LDL-C levels ≥70 mg/dL derive a clinical benefit from alirocumab treatment independent of Lp(a) levels, in those having LDL-C ~70 mg/dL alirocumab treatment provides incremental clinical benefit only when Lp(a) is at least mildly elevated (≥13.7 mg/dL) (Schwartz et al. 2021). This observation suggests that Lp(a) reduction translates into a clinical benefit, or at least allows to identify patients who may benefit from PCSK9 inhibition. It must be acknowledged that the response of Lp(a) to a PCSK9 inhibitor is highly variable and related to the size of apo(a), since each additional kringle domain is associated with an additional 3% reduction in Lp(a) (Blanchard et al. 2021).

The Prevalence of Elevated Lp(a) Levels in Individuals with Coronary Artery Disease

A large number of studies have shown that the prevalence of elevated Lp(a) in patients with CVD is higher than in the general population. As an example, the frequency of elevated Lp(a) among patients admitted to the coronary care unit was 27%, and it was even higher (32%) among patients with premature CAD (Ellis et al. 2018). Similarly, patients with relatively early onset CAD had a median Lp(a) of 29 mg/dL; levels ≥30 mg/dL were observed in half of the patients, 36.1% had an

Lp(a) 50 ≥ mg/dL, and 16.5% had Lp(a) level ≥100 mg/dL, a level that was shown to increase CV risk by about threefold (Oo et al. 2020). Likewise, among patients undergoing percutaneous coronary intervention, Lp(a) was elevated in 48% of individuals; it is worth remarking that elevated Lp(a) was observed among 45% of patients with LDL-C ≤70 mg/dL, suggesting a contribution to residual CV risk (Weiss et al. 2017).

Lp(a) in Familial Hypercholesterolemia

Familial hypercholesterolemia (FH) is a genetic condition characterized by elevated levels of LDL-C since birth and increased risk of early incident acute MI event or premature death (REF). Commonly, Lp(a) levels are higher among FH individuals than in the general population, creating a unique condition in which a lifelong exposure to two genetically elevated CV risk factors, namely Lp(a) and LDL-C, further increases the risk of MI and also predispose to aortic valve calcification (Vongpromek et al. 2015). Furthermore, a substantial proportion (~25%) of all individuals diagnosed with clinical FH were diagnosed due to high Lp(a) levels (Enkhmaa et al. 2019). The observation that FH patients carrying null mutations and Lp(a) levels >50 mg/dL have a significantly increased CV risk compared with patients carrying the same mutations and Lp(a) levels <50 mg/dL further strengthens the role of elevated Lp(a) levels in this specific, high CV risk, population (Alonso et al. 2014).

Both FH and high Lp(a) are common genetic disorders; however, both are largely undiagnosed. Direct assays for the evaluation of LDL-C, as well as LDL-C calculations, will include the cholesterol carried by Lp(a). It follows that Lp(a) concentration should be assessed in all individuals with a clinical diagnosis of FH to identify those with the highest levels and, as a consequence, the highest risk of MI (Langsted and Nordestgaard 2022); eventually, information on *LPA* variants may be useful in improving the diagnosis of FH (Chan et al. 2019). Accurate assays quantifying Lp(a)-cholesterol and the correct cholesterol content of LDL will be required to define the phenotypic differences between familial hypercholesterolemia due to elevated LDL vs. Lp(a) (Yeang et al. 2020).

The Impact of Race and Ethnicity on Lp(a) Levels and Cardiovascular Risk

The direct association between Lp(a) levels and the risk of myocardial infarction has been clearly established in populations of European ancestry (Kamstrup et al. 2008, 2009; Clarke et al. 2009). On the other hand, the heritability of apo(a) and Lp(a) levels varies across ethnicities, with, as an example, African Americans exhibiting the highest Lp(a) level despite having a lower heritability compared with

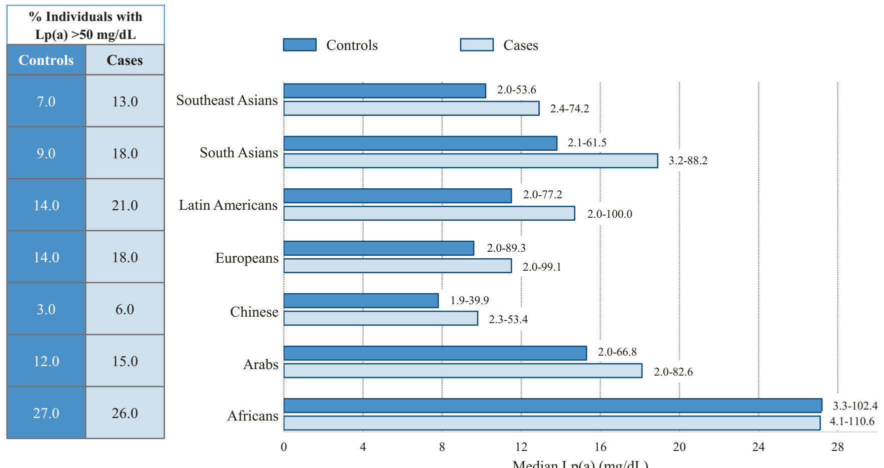

Fig. 13.3 Median levels (fifth and 95th percentile) of Lp(a) and prevalence of Lp(a) levels >50 mg/dL in individuals from diverse ancestries (in healthy individuals-CONTROLS- and patients with myocardial infarction-CASES)

Caucasians (Enkhmaa et al. 2019). Relative to Blacks, South Asians exhibit the second highest median Lp(a) level, followed by Whites, Hispanics, and East Asians; however, the causal relationship between Lp(a) and CVD extends to all racial and ethnic groups (Virani et al. 2022).

An interesting analysis of data from the INTERHEART study, involving 52 countries, showed differences in Lp(a) levels in individuals from diverse ancestries, with Africans having higher levels compared with other populations (Pare et al. 2019) (Fig. 13.3). Despite the observed differences, high Lp(a) levels were overall associated with an increased risk of MI (OR 1.48), independently of other established MI risk factors (Pare et al. 2019). When analyzed in single ethnic groups, elevated Lp(a) levels (>50 mg/dL) were associated with increased MI risk in all populations, except Arabs and Africans; in these two groups, however, the small sample size might have limited the relevance of the observation compared with other groups (Pare et al. 2019). In fact, a comparison of the association between Lp(a) and incident CV events between African Americans and Caucasians in the ARIC study showed that the hazard ratios for incident CVD and CHD were significantly higher in the highest quintile of Lp(a) (>13.5 mg/dL) in both ethnic groups; it must be acknowledged that Lp(a) levels in the highest quintile ranged from >24 to 81.7 mg/dL (median 32.1 mg/dL) in African Americans and from 13.5 to 80.3 mg/dL (median 20.4 mg/dL) in Caucasians (Virani et al. 2012). These findings need to be validated but suggest that, while Lp(a) thresholds designated on the basis of studies mainly performed in Europeans apply to different ethnic groups, they might require an adjustment for other ethnic groups having higher mean Lp(a) concentrations.

Conclusions

Although epidemiological and genetic studies have clearly established a causal role for Lp(a) in CVD, to date the evidence that reducing Lp(a) levels translates into a clinical benefit is still lacking. While substantial reductions in Lp(a) levels are required to observe a clinical benefit, new agents that potently lower Lp(a) are under clinical development. Ongoing trials will tell whether this reduction translates into reduced CVD events. A phase III study will assess the impact of Lp(a)-lowering with the antisense oligonucleotide Pelacarsen on major CV events in patients with CVD and elevated Lp(a) levels (≥70 mg/dL), with a planned follow-up of 4 years (NCT04023552).

A recent study using samples from the UK Biobank has shown that elevated Lp(a) levels are associated with an increased risk for incident CAD in individuals without a family history of heart disease, suggesting that Lp(a) evaluation may be beneficial in refining CAD risk in primary prevention patients (Finneran et al. 2021). While waiting for the results of outcome trials and looking for resolutions of major issues in the measurement of Lp(a) (Virani et al. 2022), Lp(a) assessment should be regarded as a plus in clinical practice and measured at least once in life (Mach et al. 2020; Reyes-Soffer et al. 2022), with the aim to improve risk stratification and identify individuals that might be at increased CV risk due to the presence of elevated Lp(a).

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Chapter 14
Lp(a) and Aortic Valve Stenosis, Stroke, and Other Noncoronary Cardiovascular Diseases

Anne Langsted and Pia R. Kamstrup

Introduction

High lipoprotein(a) [Lp(a)] levels are now, based on more than three decades of accumulating evidence from mechanistic, epidemiological, and genetic studies, widely recognized as an important and likely causal risk factor for ischemic cardiovascular and, in particular, coronary artery disease (CAD) (Kamstrup 2021; Reyes-Soffer et al. 2022). High Lp(a) levels in the top 10% of the concentration distribution (Fig. 14.1) associate with a two to threefold increase in risk of myocardial infarction independent of conventional risk factors (Kamstrup 2021). More recently, high Lp(a) levels, found in an estimated >1 billion individuals globally, have also been identified as a risk factor for calcific aortic valve stenosis (AVS) with risk estimates of at least the same magnitude as those found for CAD (Thanassoulis et al. 2013;

Anne Langsted and Pia R. Kamstrup contributed equally with all other contributors.

A. Langsted (✉)
Department of Clinical Biochemistry, Copenhagen University Hospital-Herlev and Gentofte, Herlev, Denmark

Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
e-mail: anne.langsted.01@regionh.dk

P. R. Kamstrup (✉)
Department of Clinical Biochemistry, Copenhagen University Hospital-Herlev and Gentofte, Herlev, Denmark

The Copenhagen General Population Study, Copenhagen University Hospital-Herlev and Gentofte, Herlev, Denmark
e-mail: pia.roerbaek.kamstrup@regionh.dk

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_14

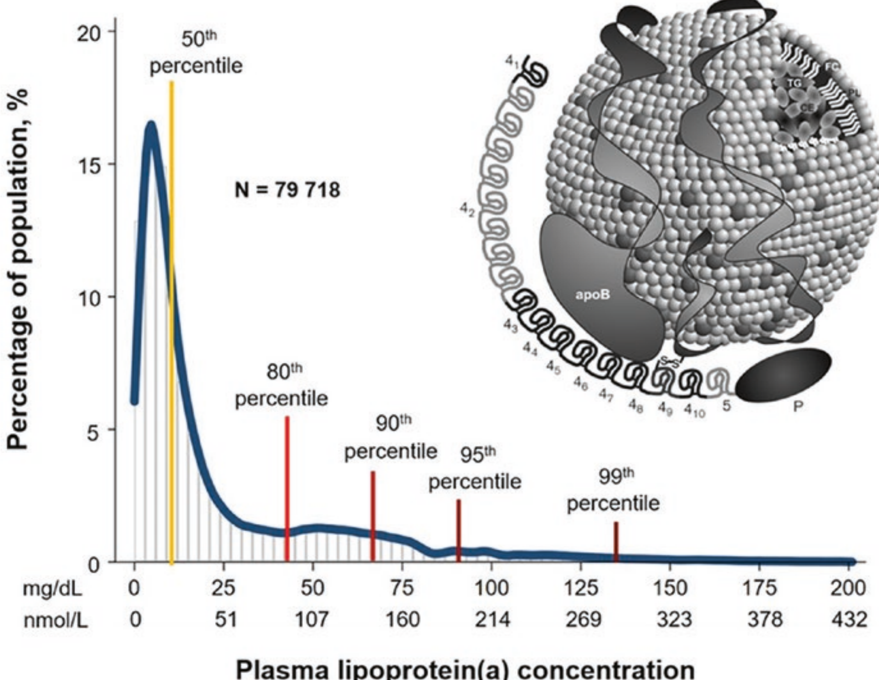

Fig. 14.1 Lp(a) concentration distribution. Plasma levels of Lp(a) (total mass and particle number) in the Copenhagen General Population Study (*N* = 79,718 White individuals of Danish descent, 118 measurements >200 mg/dL not displayed). All measurement values were calibrated to fresh sample measurements by the latex-enhanced Denka Seiken (Denka Seiken, Tokyo, Japan) immunoturbidimetric assay with traceability to an international calibrator (WHO SRM 2B). Conversion to nmol/L was done according to the following equation based on ~13,900 individuals with measurements in both mg/dL and nmol/L (Denka Seiken Roche distributed assay using different calibrations for mg/dL and nmol/L): lipoprotein(a) nmol/L = 2.18*lipoprotein(a) mg/dL–3.83. (*Adapted from Clin Chem 2021;67:154–166 with permission*)

Kamstrup et al. 2014). Several clinical guidelines on cardiovascular disease (CVD) prevention now recommend once-in-a-lifetime Lp(a) measurements in all to identify individuals at increased risk and optimize management of modifiable risk factors (Mach et al. 2020; Pearson et al. 2021).

The mounting evidence that high Lp(a) levels represent an unmet clinical need have spurred the development of potent Lp(a)-lowering drugs opening opportunities for future CVD prevention in individuals with levels above thresholds for increased risk (Reyes-Soffer et al. 2022). In this chapter, we summarize findings for Lp(a) and AVS, stroke, and other noncoronary CVD, focusing on findings from large genetic epidemiologic studies, and provide estimates for thresholds.

Aortic Valve Stenosis

Calcific AVS is a chronic, progressive disease which shares risk factors with atherosclerotic disease and is estimated to affect 3% of adults older than 75 years of age and with a steeply increasing disease burden in high-income countries (Otto and Prendergast 2014; Yadgir et al. 2020). Up to 50% of patients progress to severe disease within 2–4 years, and valve replacement represents the only treatment option for severe disease characterized by obstructive heart failure and increased risk of sudden death (Coisne et al. 2021). While familial aggregation exists for both bi- and tricuspid diseases, up until 2013, no specific genetic risk factors had been identified (Otto and Prendergast 2014). However, in a landmark study from 2013, and using a hypothesis-free genome-wide association study (GWAS) approach, the *LPA* gene was identified as the only genome-wide significant locus for the presence of aortic valve calcification (AVC) in a White European ancestry cohort ($N = 6942$) and with replication also in African American and Hispanic American cohorts (Thanassoulis et al. 2013). The *LPA* rs10455872 single nucleotide polymorphism (SNP), previously found to be strongly associated with Lp(a) levels (Clarke et al. 2009), associated with a twofold increase per allele in risk of AVC considered an early phenotype for AVS (Thanassoulis et al. 2013). Also, based on Lp(a) levels available in a subgroup ($N = 3670$), an odds ratio of 1.62 (1.27–2.06) for AVC was found per log-unit increase in genetically determined Lp(a) levels. Finally, in two cohort studies, an association with incident AVS and valve replacement was found with hazard ratios per allele of 1.7 (95% confidence interval: 1.3–2.2) and 1.5 (1.1–2.3). The combined findings of the study provided strong genetic evidence of a causal association of Lp(a) with AVC and likely also AVS.

Prior to 2013, associations of high Lp(a) levels with increased risk of AVC or AVS had only been sporadically reported in smaller epidemiological studies (Gotoh et al. 1995; Stewart et al. 1997; Glader et al. 2003; Bozbas et al. 2007). However, the 2013 GWAS study generated considerable interest in high Lp(a) levels as a possible causal risk factor for calcific AVS. Thus, in 2014, risk estimates for incident AVS at different levels of Lp(a) were reported from large general population studies. The first was a combined analysis of the historic Copenhagen City Heart Study and the contemporary Copenhagen General Population Study (Kamstrup et al. 2014). Risk of AVS was increased for Lp(a) levels in the top third of the concentration distribution (≥20 mg/dL, ≥40 nmol/L), and individuals with levels ≥90th percentile (≥65 mg/dL, ≥138 nmol/L) had a two to threefold increased risk of AVS as compared to individuals with levels <5 mg/dL ($N = 29{,}016$, Fig. 14.2). Notably, the risk estimates appeared independent of the presence or absence of CAD. On a continuous scale, a tenfold increase in plasma Lp(a) levels is associated with a hazard ratio of 1.4 (1.2–1.7) comparable to the 1.6 (1.2–2.1)-fold increase in risk found for a similar increase in genetically determined levels based on three *LPA* genotypes

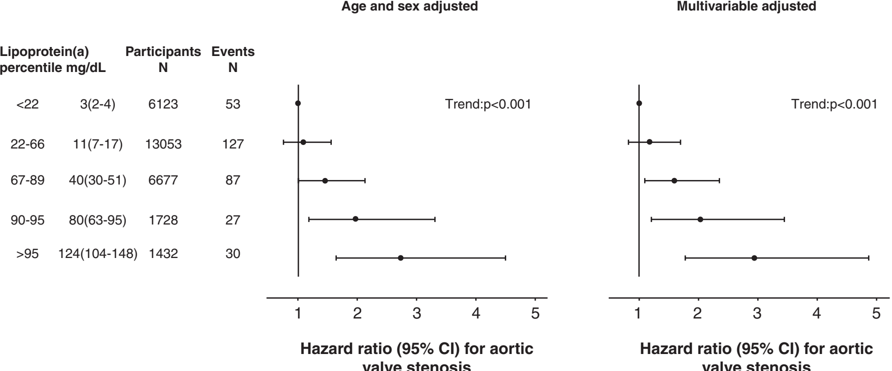

Fig. 14.2 Risk of aortic valve stenosis by Lp(a) levels. Analyses were adjusted for age, sex, total cholesterol, HDL (high-density lipoprotein) cholesterol, systolic blood pressure, smoking, and diabetes. Lipoprotein(a) in mg/dL is shown as median (interquartile range). (*Adapted from J Am Coll Cardiol 2014;63:470–7 with permission*)

(rs10455872, rs3798220, KIV-2) explaining 41% of the total variation in plasma levels. The second was from the European Prospective Investigation into Cancer (EPIC)-Norfolk study ($N = 17{,}553$), where individuals in the top third of the Lp(a) concentration distribution had a 1.6 (1.0–2.4)-fold increased risk of AVS with consistent genetic findings as hetero- and homozygous minor allele rs10455872 carriers had a 1.8 (1.1–2.9)- and 4.8 (1.8–13.2)-fold increased risk, as compared to noncarriers (Arsenault et al. 2014).

In subsequent years, additional large genetic studies provided evidence that high Lp(a) levels were at least as strong a risk factor for AVS as for CAD (Emdin et al. 2016; Gudbjartsson et al. 2019). Thus, in a study including 112,338 UK Biobank participants and using a genetic risk score based on four *LPA* SNPs associated with decreased Lp(a) plasma levels, a one standard deviation decrease in genetically determined Lp(a) levels is associated with a 37% reduced risk of AVS, compared to effect sizes of 31% for peripheral vascular disease, 29% for CAD, 17% for heart failure, and 17% for stroke (Emdin et al. 2016). Similarly, in a large case-control study of 143,087 Icelanders from 2019, genetically imputed Lp(a) levels were associated with similar risk estimates for CAD and AVS of 16–17% risk increase per 50 nM (~25 mg/dL) increase in genetically determined Lp(a) levels. The association appeared entirely mediated by increased Lp(a) levels and not by the concomitant small apolipoprotein(a) isoform size, a previous point of contention (Gudbjartsson et al. 2019).

While genetic studies have provided strong evidence for high Lp(a) levels as a cause of AVS, the pathophysiological mechanism is not fully understood. In vitro studies have, however, demonstrated osteogenic differentiation of valvular interstitial cells exposed to Lp(a) and associated pro-inflammatory oxidized phospholipids (Zheng et al. 2019), and both measurements have in AVS patient cohort studies been associated with the progression of both mild to moderate and more advanced valvular diseases (Zheng et al. 2019; Capoulade et al. 2018), and most recently also with aortic valve microcalcification in individuals without macroscopically detectable valve pathology (Despres et al. 2019), thus also pointing to a role in earlier-stage

disease. The totality of evidence pointing to high Lp(a) levels as a potentially modifiable cause of calcific AVS thus holds great promise for improved prevention of symptomatic AVS upon future availability of effective Lp(a)-lowering drugs.

Stroke

Ischemic Stroke

Lp(a) is a firmly established risk factor for myocardial infarction and AVS by proposed pathophysiological mechanisms such as atherosclerosis and thrombosis. The association of high Lp(a) levels with risk of ischemic stroke (IS) is not as firmly established as results from several studies are unclear and somewhat conflicting. Most studies did find increased risk of IS with high Lp(a) levels such as in the large prospective Atherosclerosis Risk in Communities (ARIC) study from the USA, which included both White and Black individuals with a 79% higher risk ratio for high versus low levels (Ohira et al. 2006). Another large prospective contemporary study from Denmark, the Copenhagen General Population Study (N = 46,699), found increased risk of IS per 50 mg/dL higher Lp(a) levels with a hazard ratio of 1.2 (95% CI: 1.1–1.3) in observational analyses and with genetic estimates of 1.2 (1.0–1.4) via KIV2 and 1.3 (1.1–1.5) via rs10455872, indicating a causal role (Fig. 14.3) (Langsted et al. 2019a). In support of a genetic association, the previously mentioned large UK Biobank study found an odds ratio of 0.87 (0.79–0.96) for risk of IS for one standard deviation genetically lower Lp(a) levels (Emdin et al. 2016). Also, meta-analyses of observational studies, for example, from the Emerging Risk Factors Collaboration including data from 24 studies (Emerging Risk Factors Collaboration et al. 2009) and from India including data from 41 studies (Kumar et al. 2021) on IS, find an association of high Lp(a) levels with increased risk of IS. In the Prospective Epidemiological Study of Myocardial Infarction (PRIME) study, a large prospective study from Northern Ireland and France, the association was not significant, but the point estimate indicated an increased risk of IS with high Lp(a) levels (Canoui-Poitrine et al. 2010). On the contrary, data from the Physicians' Health Study including White middle-aged males from the USA found no association between high Lp(a) levels and risk of IS (Ridker et al. 1995).

Notably, the risk estimates for IS for high Lp(a) levels or corresponding genetic variants are lower than those reported for myocardial infarction and AVS, perhaps indicating that the pathophysiology for IS might be different.

Hemorrhagic Stroke

In most cases, the pathophysiology of hemorrhagic stroke differs greatly from the causes of IS. High Lp(a) levels have previously been associated with a low risk of bleeding (Langsted et al. 2017) perhaps due to the proposed antifibrinolytic

effects of Lp(a) because of its homology with plasminogen. Most studies examining the role of Lp(a) in stroke have focused on ischemic or overall stroke as described above, and results on hemorrhagic stroke are even more mixed with both protective and pathological effects of high Lp(a) levels reported. In a study from Japan, it was found that high Lp(a) levels were associated with low risk of hemorrhagic stroke, most significantly in men with a hazard ratio of 0.44 (0.21–0.96) for highest versus lowest tertile of Lp(a) levels (Ishikawa et al. 2013). The meta-analysis from the Emerging Risk Factors Collaboration et al. (2009) including nine studies on hemorrhagic stroke found no association of Lp(a) with hemorrhagic stroke, in contrast to findings from two Chinese studies (Sun et al. 2003; Fu et al. 2020) which found high Lp(a) levels to be associated with increased risk of hemorrhagic stroke.

The complex nature and fundamentally different causes of hemorrhagic stroke compared to ischemic stroke might be one of the reasons for these highly conflicting results. Studies including subtypes of hemorrhagic stroke based on the underlying pathophysiology are needed to find meaningful associations.

Arterial Ischemic Stroke in the Young

In 2011, pediatric guidelines for cardiovascular disease risk reduction introduced screening for high Lp(a) levels in children and adolescents with a previous ischemic or hemorrhagic stroke (Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents, National Heart, Lung, and Blood Institute 2011). Several studies have examined the relationship of high Lp(a)

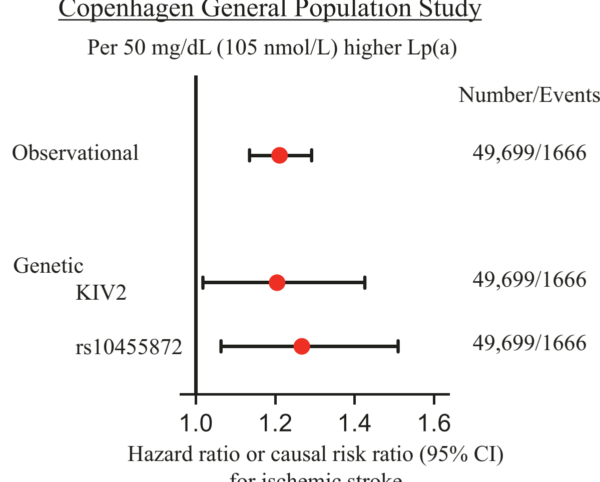

Fig. 14.3 Risk of ischemic stroke in the Copenhagen General Population Study. Age- and sex-adjusted observational odds ratio and causal genetic risk ratios by *LPA* KIV2 and *LPA* rs10455872 with 95% confidence intervals for ischemic stroke for 50 mg/dL (105 nmol/L) higher Lp(a) levels. (*Adapted from J Am Coll Cardiol. 2019;74(1):54–66 with permission from Elsevier*)

levels and risk of arterial IS in the young. A meta-analysis including 4 studies and a total of 90 events found that children with high Lp(a) levels have an odds ratio of 4.2 (2.9–6.1) for risk of arterial IS (Sultan et al. 2014). Another meta-analysis including five studies (with two studies also included in the former meta-analysis) found an odds ratio for high Lp(a) levels of 6.3 (4.5–8.7) in children with arterial IS (Kenet et al. 2010).

Of note, as arterial IS is much less prevalent in children than in adults and is often associated with underlying medical conditions, most of the studies on Lp(a) have excluded children with other risk factors which may, therefore, limit the generalizability of the study findings; however, the risk estimates are substantial and should be investigated further.

Other Noncoronary Cardiovascular Diseases

Heart Failure

The two to threefold increased risk of myocardial infarction and calcific AVS found in individuals with Lp(a) levels in the top decile is consistent with Lp(a) also being a possible risk factor for heart failure (HF), representing a major and increasing health-economic burden in aging populations (Kamstrup 2021; Heidenreich et al. 2013). In 2015, a clear stepwise association of Lp(a) levels with risk of incident HF was reported from the combined Copenhagen general population studies including >98,000 adult individuals of Danish descent (Kamstrup and Nordestgaard 2016). Lp(a) levels >90th percentile (>67 mg/dL) are associated with a 1.6–1.8-fold increased risk as compared to individuals with levels in the lower third of the concentration distribution and with comparable genetic risk estimates in support of a causal association. Notably, the association appeared largely driven by the likely causal associations of Lp(a) with myocardial infarction and/or AVS, with 63% of the Lp(a)-driven HF risk mediated by these two conditions (Kamstrup and Nordestgaard 2016). Further, a 9% population attributable risk of HF was estimated for high Lp(a) levels indicating that, given the development of future effective Lp(a)-lowering treatments, a notable decrease in HF incidence may also be achieved.

The observational association of Lp(a) levels with HF was since replicated in both the Atherosclerosis Risk in Communities (ARIC) study and in the Multi-Ethnic Study of Atherosclerosis (MESA) (Agarwala et al. 2017; Steffen et al. 2018). Findings from the large 2019 Icelandic case-control study with information on measured and genetically imputed Lp(a) plasma levels also provided additional evidence of a causal association of Lp(a) with HF (Gudbjartsson et al. 2019). Thus, a 50 nM (~25 mg/dL) increase in genetically determined Lp(a) levels is associated with a 5% increase in risk of HF. This is in addition to the more pronounced risk increases reported for CAD, AVS, and peripheral arterial disease (PAD).

Peripheral Arterial Disease

The proposed pathophysiological pathway of lipoprotein(a) through interference with fibrinolysis and thereby promoting thrombosis and the causal association with myocardial infarction could also result in arteriosclerotic or thrombotic PAD. In a prespecified analysis of the ODYSSEY OUTCOMES trial evaluating a PCSK9 inhibitor, it was found that the highest versus lowest quartile of lipoprotein(a) was associated with a hazard ratio of 2.2 (1.4–3.6) for risk of PAD and further that lowering of high lipoprotein(a) levels reduced the risk (Schwartz et al. 2020). In a general population study from Denmark, highest versus lowest tertile of lipoprotein(a) was associated with an odds ratio of 1.6 (1.3–2.0) for risk of peripheral arterial stenosis (Kamstrup et al. 2012). Further, large genetic studies examining genetically determined high lipoprotein(a) levels have also found a solid likely causal association with risk of PAD (Emdin et al. 2016; Laschkolnig et al. 2014).

Summary

Lp(a) has since its discovery been a lipoprotein particle of high interest in cardiovascular research due to a composition consistent with both proatherosclerotic and prothrombotic effects. It is now well established that high levels are associated both observationally and genetically, and therefore likely causally, with increased risk of CAD, calcific AVS, HF, IS, PAD, and mortality (Langsted et al. 2019b). The exact pathophysiology of high Lp(a) levels has not yet been elucidated and may involve, in addition to proatherosclerotic and prothrombotic effects, also proinflammatory and procalcific effects, and the exact mechanisms behind different CVD manifestations may differ.

Guidelines today are transitioning from recommending measurement of Lp(a) only in individuals at increased risk of cardiovascular disease to once-in-a-lifetime measurement in all. Currently, promising lipoprotein(a)-lowering agents are being tested, and studies will hopefully show that lowering of lipoprotein(a) will lower the risk of CVD.

Disclosures PRK reports talks and consultancies sponsored by Physicians Academy of Cardiovascular Education (PACE), Silence Therapeutics and Novartis. AL has nothing to disclose.

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Chapter 15
Lipoprotein(a) in Cardiovascular Disease: Evidence from Large Epidemiological Studies

Peter Engel Thomas, Signe Vedel-Krogh, and Børge G. Nordestgaard

Introduction

After the discovery of lipoprotein(a) by Kåre Berg in 1963, interest in lipoprotein(a) was only modest until the *LPA* gene, coding for apolipoprotein(a)—the protein unique to lipoprotein(a)—was sequenced in 1987 (Berg 1963; McLean et al. 1987) (Fig. 15.1). This significantly increased scientific interest in lipoprotein(a). However, as early general population studies failed to find a clear association between lipoprotein(a) and cardiovascular disease, interest gradually declined (Nordestgaard and Langsted 2016). Awareness of lipoprotein(a) was renewed in 2009 when genetic evidence from a Mendelian randomization analysis of two large, general population cohorts, the Copenhagen City Heart Study and the Copenhagen General Population Study, showed that high lipoprotein(a) was causally associated with myocardial infarction (Kamstrup et al. 2009). The interest in lipoprotein(a) was further complemented the same year as evidence from the Emerging Risk Factors Collaboration showed that lipoprotein(a) was continuously and independently associated with cardiovascular disease (Erqou et al. 2009) and in a large study from Clarke et al. on genetic variation in the *LPA* gene as the strongest genetic cardiovascular risk factor out of 2100 candidate genes for cardiovascular disease (Clarke et al. 2009). With the use of more accurate lipoprotein(a) assays, by accounting for regression dilution bias, and with evidence from genetic studies which largely avoid confounding and reverse causation, lipoprotein(a) was firmly established as a causal risk factor for cardiovascular disease (Nordestgaard and Langsted 2016). This led to a significant interest in developing pharmacological therapy specifically targeting lipoprotein(a),

P. E. Thomas · S. Vedel-Krogh · B. G. Nordestgaard (✉)
Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Copenhagen University Hospital, University of Copenhagen, Copenhagen, Denmark
e-mail: peter.engel.thomas@regionh.dk; signe.vedel.krogh@regionh.dk; Boerge.Nordestgaard@regionh.dk

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_15

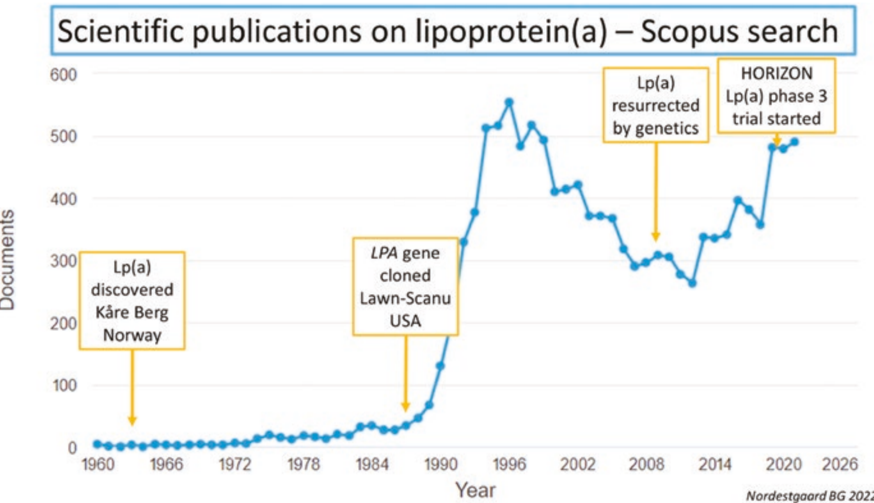


Fig. 15.1 Overview of scientific publications on lipoprotein(a) from the bibliographic database Scopus since its discovery in 1963 and up until 2021. *Lp(a)* lipoprotein(a). (Illustration by Børge G. Nordestgaard)

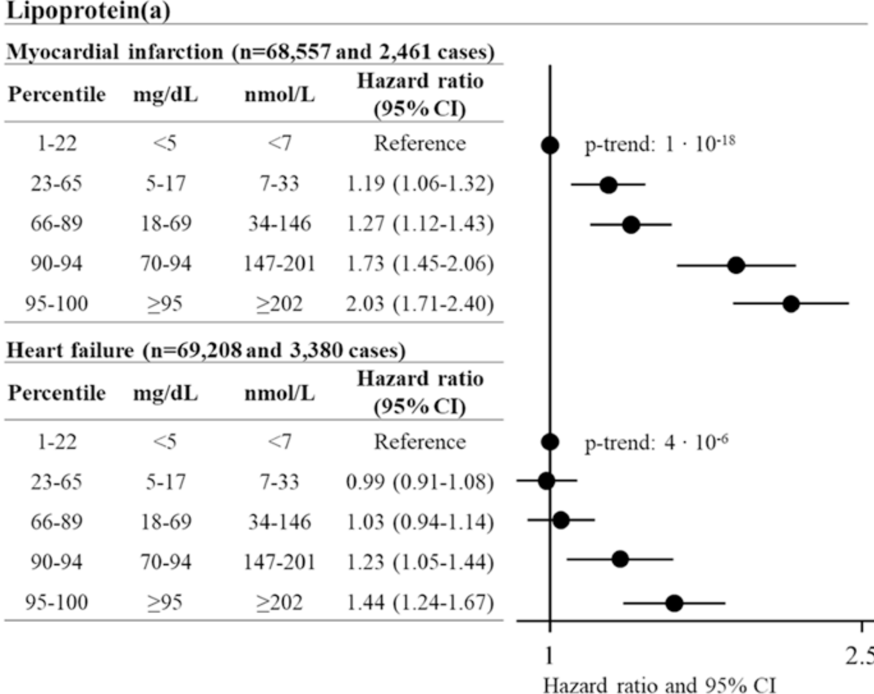
and HORIZON, the first phase 3 trial of specific lipoprotein(a)-lowering therapy, was initiated in the year 2020 (Tsimikas et al. 2020). Two drugs that can reduce plasma lipoprotein(a) by up to 90% are likewise in clinical development (Koren et al. 2022; Nissen et al. 2022).

Herein we highlight evidence from contemporary general population studies on lipoprotein(a) as a risk factor for cardiovascular disease in primary and secondary prevention cohorts, including cohorts with multiple ethnicities. We especially focus on myocardial infarction and heart failure, as evidence of lipoprotein(a) as a risk factor for ischemic stroke, aortic valve stenosis, and peripheral arterial disease is covered elsewhere in this book. The present chapter is not intended as a systematic review of all published studies but rather as an overview of important contemporary studies on elevated lipoprotein(a) as a risk factor for cardiovascular disease.

Lipoprotein(a) in Contemporary Primary Prevention Studies

Early meta-analyses of population-based prospective cohorts showed that lipoprotein levels were higher in individuals who developed ischemic heart disease than in those who did not and that individuals in the top versus bottom third of the lipoprotein(a) level distribution had a higher risk ratio of coronary heart disease (Danesh et al. 2000). In 2008, data from the prospective Copenhagen City Heart Study focused on risk of myocardial infarction in individuals with extremely high lipoprotein levels and found a three to fourfold increased risk in individuals with

extreme lipoprotein(a) levels, that is, ≥120 mg/dL (the 95th percentile) (Kamstrup et al. 2008). The study also demonstrated that the risk of myocardial infarction increased in a stepwise manner with increasing levels of lipoprotein(a). Further, when combining data from two large prospective cohorts of the adult Danish population, the Copenhagen City Heart Study and the Copenhagen General Population Study, a stepwise increase in the risk of incident heart failure was observed with increasing levels of lipoprotein(a) (Kamstrup and Nordestgaard 2016). For use specifically in this book, we have updated our former epidemiological studies based on the Copenhagen General Population Study on the association between elevated lipoprotein(a) and risk of myocardial infarction and heart failure in Fig. 15.2 (Kamstrup et al. 2008, 2009; Kamstrup and Nordestgaard 2016; Langsted et al. 2015). As can be observed, individuals with lipoprotein(a) in the 23rd–65th percentile (5–17 mg/dL or 7–33 nmol/L) have an age- and sex-adjusted hazard ratio of 1.19 [95% confidence interval (CI): 1.06–1.32] for myocardial infarction when compared with individuals with lipoprotein(a) in the 1st–22nd percentile (<5 mg/dL or <7 nmol/L). Thus, lipoprotein(a) is a risk factor at a comparatively low level with regard to myocardial infarction. However, the highest risk of myocardial infarction


Lipoprotein(a)

Myocardial infarction (n=68,557 and 2,461 cases)

Percentile	mg/dL	nmol/L	Hazard ratio (95% CI)
1-22	<5	<7	Reference
23-65	5-17	7-33	1.19 (1.06-1.32)
66-89	18-69	34-146	1.27 (1.12-1.43)
90-94	70-94	147-201	1.73 (1.45-2.06)
95-100	≥95	≥202	2.03 (1.71-2.40)

Heart failure (n=69,208 and 3,380 cases)

Percentile	mg/dL	nmol/L	Hazard ratio (95% CI)
1-22	<5	<7	Reference
23-65	5-17	7-33	0.99 (0.91-1.08)
66-89	18-69	34-146	1.03 (0.94-1.14)
90-94	70-94	147-201	1.23 (1.05-1.44)
95-100	≥95	≥202	1.44 (1.24-1.67)

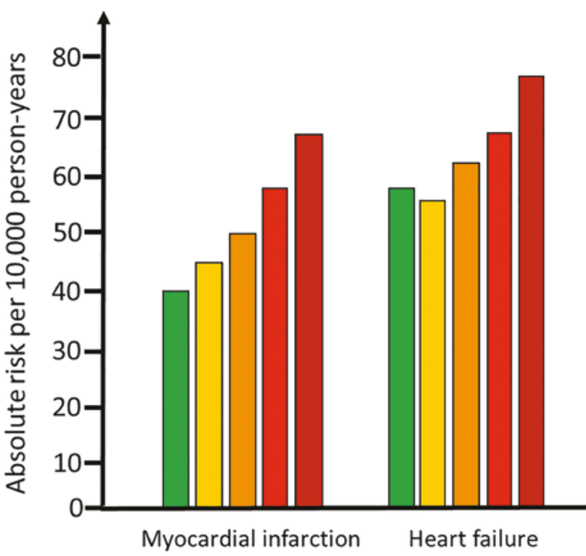
Fig. 15.2 Age- and sex-adjusted Cox proportional hazard ratios for the lipoprotein(a)-associated risk of myocardial infarction and heart failure. Based on ~69,000 individuals from the Copenhagen General Population Study. *CI* confidence interval. (Data by Børge G. Nordestgaard, Signe Vedel-Krogh, and Peter E. Thomas)

is found in individuals in the top 5% of lipoprotein(a) levels (≥95 mg/dL or ≥202 nmol/L) with a hazard ratio of 2.03 (1.71–2.40) when compared with individuals with lipoprotein(a) in the 1st–22nd percentile. For heart failure, the risk increases with levels of lipoprotein(a) above the 90% percentile; however, the highest risk is again observed in individuals in the top 5% of lipoprotein(a) levels with a hazard ratio of 1.44 (1.24–1.67) when compared with individuals with low levels of lipoprotein(a). Compared to relative risk estimates, absolute risk considers the number of events in a fixed time period without direct comparison between groups. As can be seen in Fig. 15.3, the highest absolute risks of myocardial infarction and heart failure are also seen in individuals in the top 5% of lipoprotein(a) levels with absolute risks of approximately 77 and 68 events per 10,000 person years, respectively.

Data from the UK Biobank support the findings from the Copenhagen cohorts. Like the Copenhagen studies, the UK Biobank includes individuals from the adult, general population, but the British cohort study also includes ethnicities other than White. In total, the UK Biobank includes data on 8940 (1.9%) individuals of South Asian origin, 7144 (1.6%) of Black origin, and 1435 (0.3%) of Chinese origin and thus represents the largest multiethnic prospective cohort study of lipoprotein(a) to

Fig. 15.3 Absolute risk estimates of myocardial infarction and heart failure in categories of lipoprotein(a). Based on ~69,000 individuals from the Copenhagen General Population Study. (Data by Børge G. Nordestgaard)

Plasma lipoprotein(a)			
	nmol/L	mg/dL	Percentiles
(green)	0-60	0-29	1-74
(yellow)	61-100	30-49	75-81
(orange)	101-143	50-69	82-89
(red)	144-208	70-99	90-95
(dark red)	≥209	≥100	≥96



date (Patel et al. 2021). As seen in Fig. 15.4, data from the UK Biobank illustrate the markedly heterogeneous distribution of lipoprotein(a) levels in individuals of White, South Asian, Black, and Chinese descent and a clear log-linear association between higher lipoprotein(a) levels and increased risk of atherosclerotic cardiovascular disease in the multiethnic population. In the overall study population, a hazard ratio of 1.11 (95% CI: 1.10–1.12) per 50 nmol/L higher lipoprotein(a) levels was observed. Crucially, despite the clear difference in the distribution of lipoprotein(a) levels between ethnic groups, the estimated hazard ratio for atherosclerotic cardiovascular disease per 50 nmol/L higher lipoprotein(a) was similar for Whites [hazard ratio 1.11 (1.10–1.12)], South Asian [hazard ratio 1.10 (1.04–1.16)], and Black individuals [hazard ratio 1.07 (1.00–1.15)]; there were too few Chinese for meaningful risk estimates in this group. Thus, the risk conferred by a given higher lipoprotein(a) level was broadly similar across racial groups. This is especially important for Black individuals where the median lipoprotein(a) concentration is four times higher than the median concentration in White individuals. These findings are consistent with previous cohort studies of multiple ethnicities such as the ARIC (Atherosclerosis Risk in Communities) study, the MESA (Multi-Ethnic Study of Atherosclerosis) study, and the INTERHEART study (Virani et al. 2012; Paré et al. 2019; Guan et al. 2015).

Data from the Copenhagen cohorts and the UK Biobank clearly illustrate lipoprotein(a) as a risk factor for atherosclerotic cardiovascular disease including myocardial infarction and heart failure in primary prevention studies; however, many other studies have provided crucial contributions to the understanding of lipoprotein(a) in cardiovascular disease, as reviewed previously (Nordestgaard and Langsted 2016; Kamstrup 2021). Importantly, generally observational cohort studies cannot be used to establish causality, as they may be prone to confounding and reverse causation. However, for lipoprotein(a) levels that are 80–90% genetically

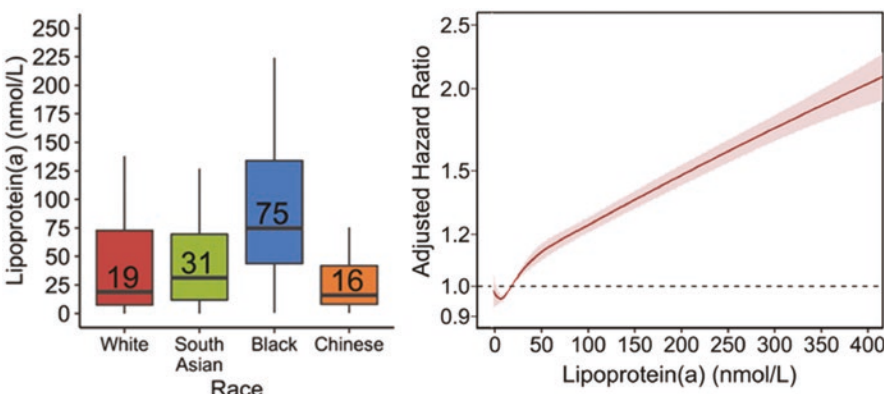


Fig. 15.4 Lipoprotein(a) concentrations according to ethnicity (left panel) and multivariable-adjusted hazard ratio for atherosclerotic cardiovascular disease according to lipoprotein(a) concentration using cubic natural splines (right panel). Based on 460,506 individuals from the UK Biobank. Left panel: Median lipoprotein(a) values, dimensions of the box capture the 25th and 75th percentiles, and whiskers capture an additional one interquartile range. (Adapted with permission from Patel et al. Arterioscler Thromb Vasc Biol. 2021;41:465–474)

Table 15.1 Sources of evidence that elevated lipoprotein(a) causes morbidity and mortality in adults in a primary prevention setting

	Case-control or cross-sectional studies	Meta-analyses of prospective observational studies	Large prospective observational studies	Large Mendelian randomization studies	Large genome-wide association studies	Randomized double-blind lipoprotein(a) lowering trials
Angina pectoris	Yes	Not examined	Yes	Yes	Yes	Not examined
Myocardial infarction	Yes	Yes	Yes	Yes	Yes	Trial ongoing
Heart failure	Yes	Not examined	Yes	Yes	Yes	Not examined
Cardio-vascular mortality	Not examined	Not examined	Yes	Yes	Yes	Trial ongoing

Table by Børge G. Nordestgaard

determined, it can be argued that even observational epidemiological studies determine causality. Nevertheless, the ascertainment of causality using direct genetic evidence for lipoprotein(a) is covered elsewhere in this book. The current evidence of lipoprotein(a) as a risk factor for atherosclerotic stenosis, myocardial infarction, heart failure, and cardiovascular mortality in primary prevention and in different types of studies is summarized in Table 15.1. To date, no randomized controlled trial has demonstrated that lowering of lipoprotein(a) results in a reduced risk of cardiovascular disease in either a primary or a secondary prevention setting. Crucially, ongoing lipoprotein(a)-lowering trials are mainly focused on secondary prevention cohorts, and much work on unraveling the role of lipoprotein(a) as a risk factor in the primary prevention setting therefore remains.

Lipoprotein(a) in Contemporary Secondary Prevention Studies

Interestingly, the role of lipoprotein(a) as a risk factor for recurrent cardiovascular events, that is, in a secondary prevention setting, long remained an area of controversy. This controversy arose from the heterogeneity of findings in secondary prevention studies, which may have been due to confounding biases such as index event bias, and a lack of individuals with high levels of lipoprotein(a) included (Boffa et al. 2018).

With the controversy regarding elevated lipoprotein(a) as a risk factor in secondary prevention in mind, Willeit et al. conducted a meta-analysis of placebo-controlled statin trials in an attempt to assess lipoprotein(a)-associated risk of cardiovascular events in patients with established cardiovascular disease (Willeit et al. 2018). Figure 15.5 shows the comparative predictive values of on-statin vs. on-placebo lipoprotein(a) for the risk of cardiovascular disease when comparing individuals with lipoprotein(a) levels of 50 mg/dL or higher with individuals with lipoprotein(a)

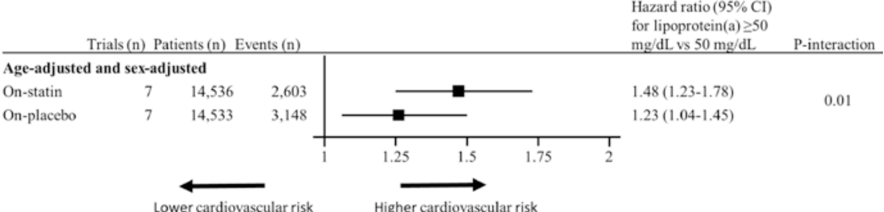


Fig. 15.5 Age- and sex-adjusted comparative predictive value of on-statin versus on-placebo lipoprotein(a) concentrations for incident cardiovascular disease. (Adapted with permission from Willeit et al. Lancet 2018;392:1311–20)

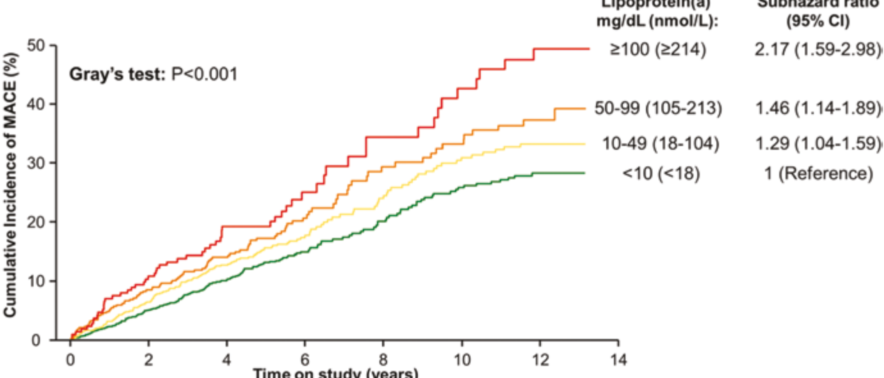


Fig. 15.6 Cumulative incidence of recurrent major adverse cardiovascular events (MACE) according to concentrations of lipoprotein(a). *CI* confidence interval. (Adapted with permission from Madsen et al. Arterioscler Thromb Vasc Biol. 2020;40:255–266)

levels lower than 50 mg/dL. The data from the seven trials included shows that higher lipoprotein(a) is positively associated with increased risk of cardiovascular events in a linear relationship independent of other cardiovascular risk factors in both patients on statin treatment and on placebo. The association of on-statin higher lipoprotein(a) with cardiovascular disease risk was stronger than for on-placebo higher lipoprotein(a), indicating that when low-density lipoprotein (LDL) cholesterol is reduced by statins, the risk conferred by elevated lipoprotein(a) becomes more important. Thus, individuals with previous cardiovascular disease and elevated lipoprotein(a) are at substantial residual risk of cardiovascular disease even while on statin treatment. Nevertheless, randomized trials can be affected by index event bias, that is, bias that may occur when the occurrence of a particular event is required for inclusion in a study (Dahabreh and Kent 2011); however, a study of 2527 individuals from the general Danish population with a history of cardiovascular disease supports the findings from the statin-trial meta-analysis (Madsen et al. 2020). Figure 15.6 shows how higher levels of lipoprotein(a) are associated with higher risk of recurrent major adverse cardiovascular events. Compared with individuals with lipoprotein(a) <10 mg/dL (18 nmol/L), the multivariable-adjusted subhazard ratios for major adverse cardiovascular events were 1.29 (95% confidence

interval: 1.04–1.59) for individuals with 10–49 mg/dL (18–104 nmol/L), 1.46 (1.14–1.89) for individuals with 50–99 mg/dL (105–213 nmol/L), and 2.17 (1.59–2.98) for individuals with ≥100 mg/dL (≥214 nmol/L). These findings were independently confirmed in two other cohorts of the Danish general population.

In conclusion, evidence from both post-hoc analyses of clinical statin trials and from general population studies of individuals with a history of cardiovascular disease suggest that elevated lipoprotein(a) is associated with an increased risk of recurrent cardiovascular disease.

Future Perspectives

Large epidemiological studies have established an association between elevated lipoprotein(a) levels and increased risk of cardiovascular disease in both primary and secondary preventive settings and in multiple ethnicities. Crucially, it cannot be deduced from these studies that lowering of lipoprotein(a) will lead to a reduction in cardiovascular morbidity or mortality. Such an effect must be demonstrated in randomized clinical trials, where one is ongoing as of the year 2022 (NCT04023552), and hopefully two further studies will follow as two new drugs already show promising results with up to a 90% lowering of lipoprotein(a) (Koren et al. 2022; Nissen et al. 2022). Results from such studies are eagerly awaited.

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Chapter 16
Lipoprotein(a) and Immunity

O. I. Afanasieva, T. I. Arefieva, M. V. Ezhov, and S. N. Pokrovsky

Introduction

Lipoprotein(a) [Lp(a)] is an atherothrombogenic lipoprotein particle that differs in its composition and physicochemical and biological properties from other lipoproteins and contains a unique apolipoprotein(a) molecule [apo(a)]. The relationship between the immune system and lipid metabolism has been evaluated for many decades. An increased blood Lp(a) concentration is a proven risk factor for atherosclerotic cardiovascular disease (ASCVD). Lawn's hypothesis about Lp(a) as a repair factor remains relevant until today (Lawn et al. 1992). Recent studies suggest participation of humoral and cell immunity in wound healing and regeneration and in inflammatory diseases (Masoomikarimi and Salehi 2022; Eming et al. 2017). An elevated Lp(a) level in long-living persons suggests possible participation of immunological factors in both the physiological and pathophysiological Lp(a) pathways (Panza et al. 2007). It is assumed that with increased life expectancy and in the presence of "inflammaging," [Inflammaging is the long-term result of the chronic physiological stimulation of the innate immune system, which can become damaging during ageing—a period of life largely unpredicted by evolution (Franceschi et al. 2018)] Lp(a) may become a factor contributing to atherosclerosis and other inflammatory diseases (Franceschi et al. 2018).

O. I. Afanasieva · T. I. Arefieva · M. V. Ezhov · S. N. Pokrovsky (✉)
National Medical Research Center of Cardiology named after E. I. Chazov MOH of Russian Federation, Moscow, Russia
e-mail: TIArefieva@cardio.ru

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_16

Lipoprotein(a) and IgG and IgM Autoantibodies (AAbs)

The production of immunoglobulins (Ig) by B cells is necessary for the recognition, neutralization, and removal of exogenous and endogenous pathogens and for maintaining homeostasis. The concept of "natural" antibodies synthesized by B1 cells with specificity to alien and native proteins was first proposed in 1908 by Ehrlich (Piro et al. 2008). Natural IgM antibodies are encoded by the germline cells, and they are present in the umbilical cord blood of newborns. It is assumed that the level of natural IgM antibodies is maintained constant throughout life (Holodick et al. 2017). The main biological functions of natural IgM are removal of apoptotic cells, protection from infection, and maintenance of tissue homeostasis (Reyneveld et al. 2020; Wang et al. 2016). The protective effect of IgM AAbs to oxidized LDL (low-density lipoprotein) (oxLDL) produced by B1 cells has been described in several studies and literature reviews (Tsimikas et al. 2012; van den Berg et al. 2018; Pattarabanjird et al. 2021). The presence of circulating Lp(a)-containing immune complexes in the plasma of patients with coronary heart disease (CHD), healthy donors, and patients with autoimmune diseases has been reported in several studies. Most of the immune complexes found in the plasma of healthy donors contained IgM AAbs against Lp(a), unlike patients with CHD (Wang et al. 2003; Sabarinath and Appukuttan 2015; Klesareva et al. 2016).

Recently, we have shown that the levels of IgM AAbs against Lp(a) were higher in patients without atherosclerosis or non-stenosing lesions of the coronary arteries (Afanasieva et al. 2016b). Such a protective function of these IgM AAbs was also present in patients with severe hypercholesterolemia (Klesareva et al. 2018). In a retrospective study of 1228 patients, the lower the IgM level of Lp(a) AAbs and the higher the concentration of Lp(a), the more vascular beds there were with stenosing atherosclerotic lesions (Tmoyan et al. 2021).

The autoimmune theory of atherosclerosis was formulated by Klimov more than 40 years ago. He showed that modified lipoproteins acquire autoantigenic properties and trigger an immune response to the "altered self" (Klimov 1990). The role of autoantigens is played by modified LDL, as well as lipoproteins containing oxidized phospholipids (Virella and Lopes-Virella 2008). Elevated plasma levels of IgG AAbs to oxLDL are associated with angiographically verified coronary atherosclerosis and progression of carotid lesions (Salonen et al. 1992). Previously, a direct relationship between the level of IgG AAbs against Lp(a) and the number of affected coronary arteries was demonstrated (Afanas'eva et al. 2014). The content of IgG AAbs against MDA (malondialdehyde)-LDL in the upper quartile was associated with the risk of cardiovascular events at a 10-year follow-up (Prasad et al. 2017). However, the role of Lp(a), as well as oxLDL, as possible specific autoantigen for B2 cells remains controversial (Ravandi et al. 2011). Nevertheless, studies aimed at using immunoglobulins specific to oxidized epitopes present on lipoproteins' and apoptotic cells' surfaces for the treatment of ASCVD are in progress (de Vries et al. 2021; Pluijmert et al. 2021; Ståhle et al. 2020).

Lp(a), such as LDL-like particles, also could be affected by modification of their protein and/or lipid compounds; such modifications activate humoral immune responses and create AAbs formation. Lp(a) AAbs immune complexes removed by macrophages can be transferred to foam cells.

The IgM and IgG antibody classes against Lp(a) detected in human serum appear to have not only different origins but also different functions. Natural IgM implies an evolutionary advantage to neutralize Lp(a) and to eliminate it. The appearance of autoantibodies of different IgG subclasses indicates the activation of adaptive immunity, which perceives Lp(a) as the antigen, and causes subsequent development of inflammatory reactions.

Lipoprotein(a) and Innate Immunity Cells

Monocytes and macrophages play a critical role in innate immunity (Libby et al. 2013) and have been the subject of numerous studies in connection with Lp(a). Lp(a) was detected in macrophage cell-rich areas of atherosclerotic plaques in humans according to morphology and immunohistochemistry studies (Sotiriou et al. 2006). On the other hand, individuals with elevated Lp(a) level exhibit enhanced accumulation of peripheral blood mononuclear cells in the arterial wall compared to individuals with normal levels of Lp(a) (van der Valk et al. 2016). Apo(a) stimulates the production of reactive oxygen species and matrix metalloproteinase-9 by collagen-adherent monocytes, and this effect was inversely associated with the molecular weight of apo(a) (Sabbah et al. 2019). Apo(a) also caused increased secretion of IL-8 by macrophages of the THP-1 and U-937 cell lines (Scipione et al. 2015). Monocytes isolated from subjects with elevated Lp(a) demonstrated an enhanced cell surface expression of chemokine receptors, adhesion molecules, and scavenger receptors (CCR7, CD62L, CD11b, CD11c, CD29, CD36, SR-A). Apo(a) upregulates the expression of the β2-integrin Mac-1 (CD11b/CD18), thereby facilitating cell adhesion and migration capacity. Several signaling cascades leading to altered gene expression profiles were found to contribute to Lp(a)-induced monocyte chemotactic activity (Scipione et al. 2015; Dzobo et al. 2022).

Besides displaying an activated and proinflammatory phenotype, monocytes isolated from individuals with elevated Lp(a) exhibited an increased secretion of proinflammatory cytokines (IL-1β, IL-6, TNFα) and a decrease in the anti-inflammatory cytokine IL-10 after stimulation via toll-like receptors. OxPLs associated with apo(a) as potent danger-associated molecular patterns (DAMPs) could be responsible for these effects (Koschinsky and Boffa 2022).

Apo(a) antisense treatment resulted in downregulation of proinflammatory gene expression in monocytes, including interferon (IFN)α, IFNγ, and toll-like receptor (TLR) pathways, and subsequent changes in monocyte phenotype and function, that is, a reduction in chemokine receptors CCR2 and CX3CR1 and transendothelial migratory capacity (Stiekema et al. 2020).

The number of circulating monocytes in apo(a) transgenic mice was four times higher than in wild-type mice and remained elevated for 3 weeks after Ca^{2+}-induced vascular damage (Huang et al. 2014). Also, Lp(a) affects the maturation of monocytes in humans (Schnitzler et al. 2020).

Monocytes are divided into three subpopulations, depending on the content of CD14 and CD16 surface markers, classical CD14++CD16−, intermediate CD14++CD16+, and nonclassical CD14+CD16++, while the latter two populations have the most pronounced proinflammatory and profibrotic potential. The participation of circulating monocytes in atherogenesis has been proven (Vergallo and Crea 2020), but the contribution of various subpopulations of monocytes to chronic inflammatory states is currently under discussion (Yang et al. 2014; Ożańska et al. 2020).

The high content of CD16+ monocytes is associated with unstable atherosclerotic plaques in the coronary arteries (Kashiwagi et al. 2010) and predicts the risk of cardiovascular events (Rogacev et al. 2012). In CHD patients, an increased content of intermediate monocytes CD14++CD16+ occurs with hyperlipoproteinemia(a) (Krychtiuk et al. 2015a), atherogenic dyslipidemia (Krychtiuk et al. 2015b), and dysfunctional high-density lipoproteins (Krychtiuk et al. 2014). The association of elevated Lp(a) concentration with absolute and relative content of CD14+CD16++ was shown in a retrospective study (Afanasieva et al. 2021). Since the function of this subpopulation is to remove "cellular debris," it is assumed that it contributes to elimination of excess Lp(a).

Neutrophil granulocytes are the largest population of circulating phagocytizing leukocytes capable of synthesizing a wide range of substances. Neutrophils and "neutrophil extracellular traps" (NETs) formed by them were found in atherosclerotic plaques of laboratory animals and humans (Afanasieva et al. 2021). NETs stimulate the production of IL-1 by macrophages and activate IL-17-producing T-helpers (Th17) in apoE-deficient mice, contributing to inflammation in the vessel wall (Döring et al. 2017). There are no data on the effect of Lp(a) or apo(a) on the formation of neutrophil traps. The absolute number of neutrophils and the neutrophil-lymphocyte index, as well as the concentration of Lp(a), was significantly higher in patients with stenosing atherosclerosis of various vascular beds (Tmoyan et al. 2021). The evaluation of the effect of Lp(a) on neutrophil activation is a promising avenue of further research.

The Role of Lipoprotein(a) and Proinflammatory Status in ASCVD Pathogenesis

Data on the association of increased Lp(a) concentration with systemic inflammation and its markers are ambiguous (Pirro et al. 2017). The risk of cardiovascular events associated with Lp(a) was significantly higher in the presence of "proinflammatory" genotype IL-1 (Naka et al. 2018) or elevated C-reactive protein level (Puri et al. 2020).

A higher lymphocyte count is associated with a higher apoB level; Lp(a) was inversely associated with basophil count in men but not in women according to a population study with 417,132 participants (Tucker et al. 2021). Low molecular weight apo(a) phenotype, reduced lymphocyte count, and increases in neutrophil granulocytes potentiated the risk of CHD in patients with type 2 diabetes (Suzuki et al. 2013).

The combination of a higher absolute monocyte count (>0.54 × 10^9 cells/mL) with elevated Lp(a) (≥30 mg/dL) is associated with higher risk of major adverse cardiovascular events (MACE) in patients with premature CHD manifestation (Afanasieva et al. 2022) (Fig. 16.1). An increase of Lp(a) concentration and the percentage of CD14++CD16+ monocytes potentiated risk of multivessel coronary disease (Afanasieva et al. 2021; Filatova et al. 2022) (Fig. 16.2).

A lower level of IgM AAbs against Lp(a) is negatively correlated with the concentration of sCD25 [the soluble form of the IL-2 receptor and a surrogate marker of T-cell activation (Brusko et al. 2009)] and associated with stenosing coronary atherosclerosis (Afanasieva et al. 2016b). This fact may serve as a confirmation of participation of both Lp(a) and T-cells in atherogenesis and also the immunomodulatory ability of IgM AAbs against Lp(a) (Wang et al. 2016).

Systemic inflammation accompanies age-related changes in lymphocyte subpopulations (Thomas et al. 2020). In patients with ASCVD, the number of naïve lymphocytes, including regulatory cells, decreases with age, while the level of effector populations, that is, Th1 and Th17, remains constant (Filatova et al. 2021). T-Lymphocytes with predominating Th1 are detected in atherosclerotic plaques (Saigusa et al. 2020). Th17, a subpopulation of CD4+ lymphocytes producing IL-17, also has a proatherogenic effect. Th17 cells participate in the immune response against their own and alien antigens by attracting myeloid cells to a place of inflammation, activating lymphocytes and secreting proinflammatory cytokines (Gao et al. 2010; Park et al. 2005). On the contrary, regulatory T-cells have anti-inflammatory activity and inhibit atherogenesis (Albany et al. 2019). Thus,

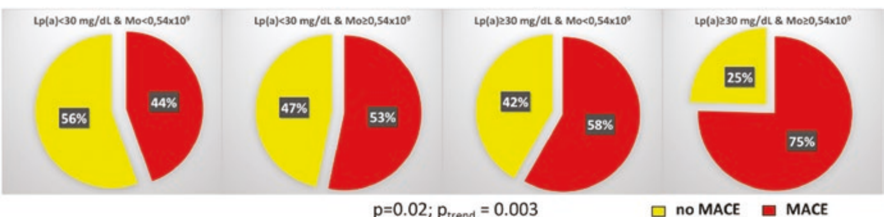

Fig. 16.1 The proportion of major adverse cardiovascular events (MACE) in patients with premature coronary heart disease depending on blood monocyte count and lipoprotein(a) concentration. Two-hundred *adult patients with early* coronary heart disease *manifestation (before 55 years in men and 60 years in women) were enrolled, median follow-up 12 years. MACE, nonfatal myocardial infarction, ischemic stroke, coronary artery bypass grafting, and hospitalization for unstable angina* (Afanasieva et al. 2022)

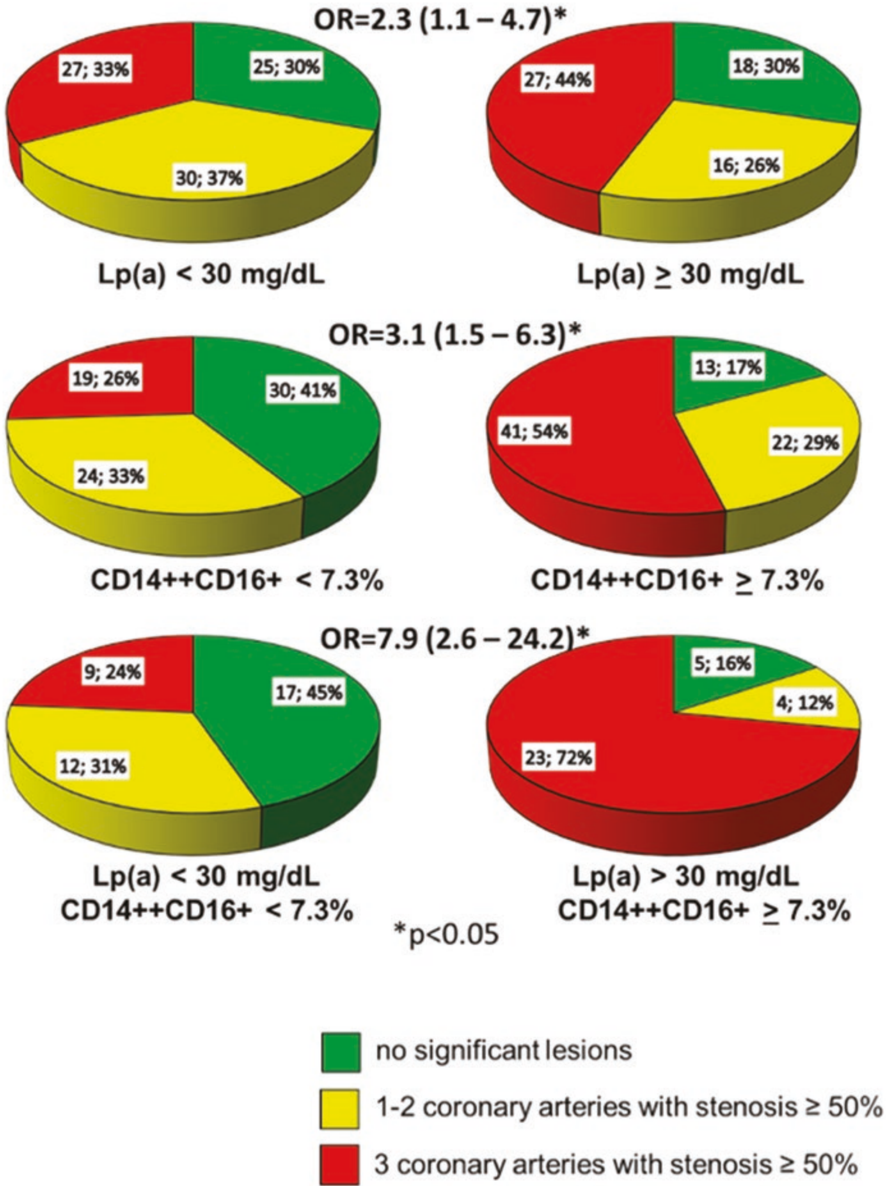

Fig. 16.2 Association of lipoprotein(a), CD14++CD16+ intermediate monocyte subpopulation, and their association with coronary atherosclerosis severity (n = 150). *Odds ratio (OR) of triple vessel disease* vs *no significant, and 1–2-vessel disease was calculated according to logistic regression analysis adjusted for age, sex, type 2 diabetes, and hypertension* (Afanasieva et al. 2021)

age-related deficiency of regulatory cells and a shift of the immune balance toward effector populations may contribute to atherosclerosis progression.

Activation and increased amounts of Th17 are related to the progression of atherosclerosis and risk of coronary events (Liuzzo et al. 2013). The ratio of circulating Treg/Th17 is reduced in patients with severe coronary atherosclerosis (Potekhina et al. 2015). The concentration of Lp(a) is not associated with the content of various T-cell subpopulations (Afanasieva et al. 2016a, b). However, an increased content of circulating Th17 (% of CD4+ lymphocytes), as well as a reduced content of Treg or IL-10 CD4+-producing cells along with Lp(a) concentrations above 12 mg/dL, is associated with severe coronary atherosclerosis (Afanasieva et al. 2016b) and carotid atherosclerosis progression (Afanasieva et al. 2016a). Thus, the increased concentration of Lp(a) and proinflammatory status with some shifts in immunity could potentiate atherosclerosis progression.

Lipoprotein(a) as a Carrier of Inflammatory Mediators

Differences in the physicochemical and immunochemical properties of LDL and Lp(a) have been noted for a long time (Zawadzki et al. 1988). The apo(a) moiety has a binding site for oxidized phospholipid (oxPL) that determines its proinflammatory effects on immune cells (Koschinsky and Boffa 2022).

Proteomic analysis shows that Lp(a) may serve as a carrier of many protein molecules, and their spectrum differs in Lp(a) and LDL (Bourgeois et al. 2020a; von Zychlinski et al. 2011, 2014). These proteins can participate in the processes of oxidation, cell proliferation and intercellular interactions, immunomodulation and activation of the complement system, and blood clotting (Bourgeois et al. 2021).

Such a variety of proteins can provide Lp(a) particles with the ability to participate in the response to injury or damage. Possible ways that Lp(a) participates in activation of the immune system via its plasma components are shown in Fig. 16.3.

The complement components C3 and C4 associated with Lp(a) could determine the interaction of Lp(a) with innate and acquired immunity. The complex of Lp(a) with α2 macroglobulin can interact with low-density lipoprotein receptor-related protein 1 (LRP-1) and can not only contribute to the internalization of Lp(a) with high molecular weight isoforms of apo(a) (März et al. 1993) but also induce the migration of myeloid cells, such as monocytes and neutrophils.

Lp(a) constitutes the main pool of lipoprotein-associated proprotein convertase subtilisin/kexin type 9 PCSK9 (Tavori et al. 2016). There is evidence of the modulating effect of PCSK9 on cell immunity (Liu and Frostegard 2018; Kim et al. 2019). Also, PCSK9 can regulate the number of CD36 and LRP-1 receptors (Shapiro et al. 2018), which are expressed by hematopoietic cells, participating in the processes of hemostasis, inflammation, and tissue regeneration. The binding of PCSK9 to CD36 (Qi et al. 2021) can be recognized as a "danger signal" of innate immunity (Silverstein 2021).

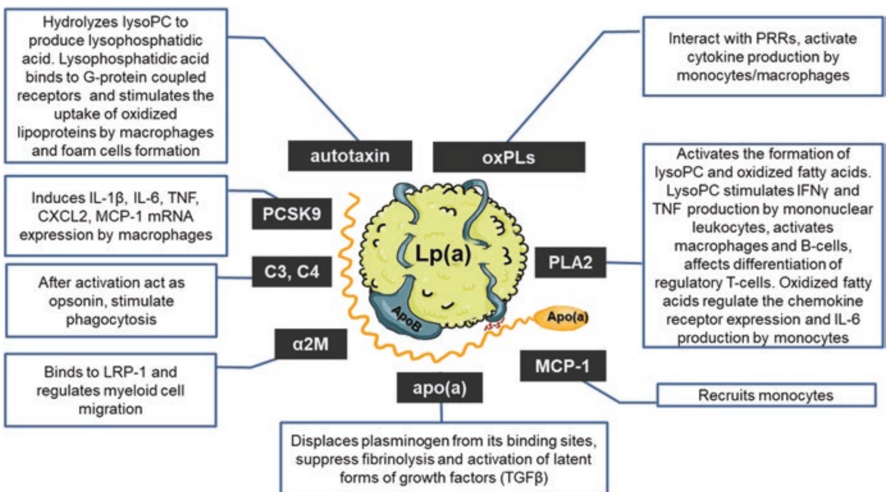

Fig. 16.3 Possible mechanisms of lipoprotein(a) contribution to immune cell activation. *lysoPC* lysophosphatidylcholines, *LPARs* LPA receptors or G-protein-coupled receptors, *IL-1β* interleukin 1β, *IL-6* interleukin 6, *TNF* tumor necrosis factor, *CXCL2* chemokine (C–X–C motif) ligand 2, *MCP-1* monocyte chemoattractant protein 1, *mRNA* messenger RNA, *LRP-1* low-density lipoprotein receptor-related protein 1, *TGFβ* transforming growth factor beta, *IFNγ* interferon γ, *PRRs* pattern recognition receptors, *oxPL* oxidized phospholipids, *PLA2* phospholipase A2, *α2M* alpha-2-macroglobulin, *PCSK9* proprotein convertase subtilisin/kexin type 9, *C3 and C4* complement components 3 and 4, and *apo(a)* apolipoprotein(a)

Lp(a) as a possible carrier of autotaxin and a source of lysophosphatidic acid is associated with calcification and aortic valve stenosis (Bouchareb et al. 2015; Bourgeois et al. 2020b). The lysophosphatidic acid participates in the differentiation and homing of T-lymphocytes (Zhang et al. 2012; Knowlden and Georas 2014). Both facts suggest another possible mechanism of Lp(a) action on the immune system.

An association of MCP-1 with Lp(a) via oxidized phospholipids of Lp(a) has been described (Wiesner et al. 2013). The attachment of Lp(a) containing MCP-1 at the site of injury can lead to increased recruitment of monocytes. Thus, proteins associated with the Lp(a) particle as well as oxPL may explain its proinflammatory properties.

Many properties of Lp(a), as well as its biological roles, remain a mystery despite more than 50 years of research. Lp(a) is able to carry affected areas not only the cholesterol necessary for the synthesis of new cells but also biologically active components that attract phagocytes of the innate immune system. It can be assumed that the original role of Lp(a) as a factor in damage repair and transport systems has largely been lost at the present time. An increased concentration of Lp(a) set against the background of genetic, epigenetic, and environmental variables has become a powerful risk factor for atherosclerotic cardiovascular diseases. We designed an immunosorbent for specific Lp(a) apheresis and proved that specific Lp(a), but not

LDL, removal by extracorporeal treatment can lead to stabilization and even regression of atherosclerotic lesions in coronary and carotid arteries (Pokrovsky et al. 2016, 2020). This study was the first direct clinical observation and confirmation of Lp(a) atherogenicity in humans (Pokrovsky et al. 2017). The elucidation of molecular and cellular mechanisms of Lp(a) involvement in inflammatory remodeling of the arterial wall engaging the Lp(a) immunity axis is a promising direction for the development of new therapeutic approaches.

Lp(a) is an extremely interesting polymolecular complex, and as we learn more about it, it is clear the less we understand about its enormous functional range and its capacity to interact with and influence important pathways, such as immunity, inflammation, thrombosis, and oxidation.

Acknowledgements The authors are grateful to Professor Gilbert Thompson for his help in proofreading text of the manuscript.

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Chapter 17
When Should We Measure Lipoprotein(a)?

Karam Kostner

Introduction

Lipoprotein(a) was originally described by K. Berg in 1963 as a genetic variant of β-lipoprotein (Kostner and Kostner 2017). Evidence from large observational and epidemiological studies support causality of Lp(a) as one of the strongest risk factors for atherosclerotic cardiovascular disease (ASCVD) and calcific aortic valve disease (CAVD). These are further supported by genome-wide association and Mendelian randomisation studies (Kostner et al. 2018). Levels above 50 mg/dL are considered elevated and seen in up to 20% of the population. Over the last 10 years, there has been much discussion about when to measure Lp(a) and how to treat it (Kostner et al. 2013). It is generally accepted to measure Lp(a) in individuals with premature CV disease when traditional risk factors do not account for this. Lp(a) is also often measured to reclassify risk in intermediate-risk individuals, where elevated levels lead to more aggressive treatment of other risk factors. Imaging modalities such as coronary calcium scores are often used in conjunction with traditional and emerging plasma markers to estimate risk. Several phase II and III trials with antisense and Si RNA-targeted therapies are currently under way and will help us understand whether Lp(a) lowering in and of itself reduces CV risk. With the availability of effective therapies, it will be possible to define groups who benefit from these therapeutic interventions. The cost-effectiveness of routine screening and testing for Lp(a) also remains to be shown.

K. Kostner (✉)
Department of Cardiology, Mater Hospital and University of Queensland, Brisbane, Australia
e-mail: k.kostner@uq.edu.au

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_17

Lp(a) and ASCVD Risk Assessment

Lp(a) levels are relatively stable over a lifespan as they are mainly genetically determined which is why a single measurement of serum Lp(a) is sufficient for most patients unless a secondary cause is suspected or a specific treatment is started to reduce its concentration. Availability and reimbursement of cost-effective methods to measure Lp(a) as well as standardisation of assays are important and are discussed in different chapters of this book. It is generally more practical and cost-effective to measure Lp(a) concentrations instead of its genetic determinants. Lp(a) measurement may be considered in both primary and secondary preventions. In children with familial hypercholesterolemia (FH), for example, Lp(a) is a better predictor of CV disease in family members than LDL (Zawacki et al. 2018).

In primary prevention, focus should be directed towards absolute CV risk assessment, where patients with elevated Lp(a) are treated more aggressively for traditional risk factors such as LDL, especially if they are in an intermediate-risk group (Verbeek et al. 2018). The availability of imaging methods such as calcium scoring by CT and plaque assessment by CT, MRI and ultrasound has improved CV risk assessment and is often used in conjunction with lipid risk factors such as Lp(a). In secondary prevention, elevated Lp(a) is a driver of residual CV risk. The Justification for the Use of Statins in the JUPITER study (Khera et al. 2014) supported the premise that Lp(a) is a significant independent contributor to residual risk. This is further supported by data from the Atherothrombosis Intervention in Metabolic syndrome with low HDL/high triglycerides: Impact on Global Health outcomes (AIM-HIGH) study (Albers et al. 2013) and a recent meta-analysis (Willeit et al. 2018).

Recent outcome studies with proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors have underlined the importance of Lp(a) measurement. In the Further Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk (FOURIER) trial, reduction in risk of major acute coronary events (MACE) with evolocumab was associated with the baseline and change in Lp(a) levels (O'Donoghue et al. 2019). In the Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab (ODYSSEY OUTCOMES) trial, reduction in risk of total cardiovascular events with alirocumab was also associated with baseline and change in Lp(a) levels (Szarek et al. 2020). Reduction in risk of major adverse limb events (MALE) with alirocumab was also associated with baseline and change in Lp(a) levels (Schwartz et al. 2020). These trials support the conclusion that elevated Lp(a) is a major driver of residual risk. Although large cardiovascular outcome trial data does not currently exist to guide Lp(a) therapeutic intervention, indicators of significant increased risk, including multivessel disease, PAD (peripheral artery disease), premature disease onset, familial hypercholesterolaemia (FH), diabetes, renal disease and recurrent presentations with acute coronary syndrome (ACS), will likely be considered as clinical indicators for consideration of agents specifically targeting Lp(a) and

Table 17.1 Indications for measurement of Lp(a)

Measurement of Lp(a)
(1) Should be considered in adults to assess or stratify ASCVD risk in those with the following clinical features: a personal history of premature ASCVD (<60 years), family history of premature ASCVD, family history of high Lp(a) (>200 nmol/L), familial hypercholesterolaemia, significant renal impairment and early-onset calcific aortic stenosis (<60 years)
(2) Should be considered in those with intermediate 10-year ASCVD risk (5–15%) when classical risk algorithms are used such as the Framingham risk score, the PROCAM risk score, the ESC Heart Score or the Australian and New Zealand risk calculator, if it allows patients to be re-stratified into a higher-risk category if Lp(a) is elevated above >200 nmol/L, which in turn leads to more intensive management of treatable risk factors, especially LDL cholesterol
(3) Should be considered in those with suboptimal lowering of low-density lipoprotein cholesterol (LDL-C) despite adherence to guideline-recommended treatment
(4) Should be considered in those with recurrent or progressive ASCVD despite of optimally treated plasma LDL-C concentrations
(5) Should be considered in children and adolescents with familial hypercholesterolaemia, premature ASCVD, a first-degree relative with significantly elevated Lp(a) (>200 nmol/L) and a family history of premature ASCVD

already lead many clinicians to try to achieve very low LDL targets with statins, ezetimibe and PCSK9 inhibitors. Measurement of Lp(a) is discussed as follows and is summarised in Table 17.1:

1. Measurement of Lp(a) should be considered in adults to assess or stratify ASCVD risk in those with the following clinical features: a personal history of premature ASCVD (<60 years), family history of premature ASCVD, family history of high Lp(a) (>200 nmol/L), familial hypercholesterolaemia, significant renal impairment and early-onset calcific aortic stenosis (<60 years).
2. Measurement of Lp(a) should be considered in those with an intermediate 10-year ASCVD risk (5–15%) when classical risk algorithms are used such as the Framingham risk score, the PROCAM risk score, the ESC Heart Score or the Australian and New Zealand risk calculator, if it allows patients to be re-stratified into a higher-risk category or if Lp(a) is elevated above >200 nmol/L, which in turn should ultimately lead to more intensive management of treatable risk factors, especially low-density lipoprotein cholesterol (LDL-C).
3. Measurement of Lp(a) should be considered in those with suboptimal lowering of LDL-C despite adherence to guideline-recommended therapy.
4. Measurement of Lp(a) should be considered in those with recurrent or progressive ASCVD despite optimally treated plasma LDL-C concentrations.
5. Measurement of Lp(a) should be considered in children and adolescents with familial hypercholesterolaemia, premature ASCVD, a first-degree relative with significantly elevated Lp(a) (>200 nmol/L) or a family history of premature ASCVD.

Recommendations from International Guidelines

Even though most major international guidelines recognise that Lp(a) is a risk enhancing factor, there is still no unanimous agreement as to when to measure Lp(a) and how to deal with increased Lp(a) values. The reasons for this are that few commonly accepted assays and reference standards exist, there is a lack of effective medications available to lower Lp(a) and apart from LDL apheresis, therapeutic interventions to lower Lp(a) have not yet shown a reduction in MACE. Traditionally, levels >30 mg/dL were considered elevated, with thresholds for inclusion into outcome trials generally higher (>50 mg/dL). Table 17.1 shows risk thresholds for different Lp(a) levels.

The European Atherosclerosis Society and European Cardiology Societies, however, likely underestimate the importance of elevated Lp(a) as they focus only on people with extremely elevated levels (>180 mg/dL or >430 nmol/L) who they suggest may have a lifetime risk of ASCVD equivalent to that of heterozygous FH (Mach et al. 2019). They do recommend that Lp(a) measurement be considered at least once in each adult person's lifetime to assist with risk stratification, particularly in those considered at moderate or higher risk (Mach et al. 2019). The HEART-UK consensus statement on Lp(a) also supports the measurement of Lp(a) levels in patients with a personal or family history of premature ASCVD, those with FH or other genetic dyslipidaemias (such as familial combined hypercholesterolaemia) or early-onset ASCVD and patients with first-degree relatives who have significantly elevated Lp(a) (>200 nmol/L) levels. The statement suggests that the cardiovascular risk conferred by Lp(a) is determined by its serum concentration, with 32–90 nmol/L equivalent to minor risk, 90–200 nmol/L to moderate risk and 200–400 nmol/L to high risk, with concentrations >400 nmol/L equivalent to very high risk (Kostner et al. 2018), Table 17.2.

The National Lipid Association (NLA) suggests that the 80th percentile in predominantly Caucasian US populations is ~100 nmol/L and ~150 nmol/L in African Americans, although it is unclear whether different risk thresholds should be applied (Wilson et al. 2019). The American Heart Association (AHA) and American College of Cardiology (ACC) recognise elevated Lp(a) as a 'risk-enhancing factor' in the development of ASCVD, with levels ≥125 nmol/L (≥50 mg/dL) considered high risk (Grundy et al. 2018). Other groups, including the Canadian Cardiovascular Society and the Mighty Medic Group, suggest that Lp(a) might aid risk assessment in patients at high risk or with premature CVD/CAD, with Lp(a) levels <30 mg/dL considered normal (Anderson et al. 2016). Two International Classification of

Table 17.2 Risk thresholds for Lp(a) concentration (adapted from Heart UK consensus statement)

ASCVD risk	Lp(a) level, (nmol/L)	Lp(a) level, (mg/dL)	Percentile of population
Moderate	100–200	40–90	80–95th
High	200–400	90–180	95–99th
Very high	>400	>180	99th

Source: Cegla et al. (2019)

Diseases (ICD)-10 codes have been added to justify Lp(a) testing, E78.41 = elevated Lp(a) and Z83.430 = Family History of elevated Lp(a).

Synopsis (Authors' Recommendations)

Knowledge of Lp(a) levels is particularly valuable in reclassification of patients at intermediate risk of ASCVD, as assessed by established risk algorithms, especially if it leads to more aggressive therapy of other risk factors such as LDL. Lp(a) should also be measured in individuals with a personal or family history of premature ASCVD (or calcific aortic valve stenosis) and familial hypercholesterolaemia (FH) and in those with recurrent vascular events despite optimal LDL lowering. Information on Lp(a) levels may guide more aggressive treatment of conventional risk factors or lead to assessment of subclinical atherosclerosis with newer imaging methods such as CT.

The value of cascade testing first-degree relatives of an index case with very high Lp(a) has not been demonstrated. However, it could help define and consolidate the family history of ASCVD and improve adherence to existing therapies in secondary prevention, as well as adherence to healthy lifestyle and behaviour in primary prevention in family members. Elevated Lp(a) with a co-existent polygenic hypercholesterolaemia or familial combined hyperlipidaemia may mimic FH and should always be considered in patients who return a negative genetic test for FH. Finally, results from large clinical trials with Lp(a)-lowering agents that are currently underway will likely have an impact on Lp(a) measurement and likely provide us with effective therapies for this atherogenic lipoprotein.

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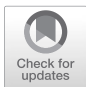
Chapter 18
Measurement of Lipoprotein(a) in the Clinical Laboratory



David Sullivan, Catherine Woolnough, Nimalie Perera, Jay Ramanathan, and Tony Badrick

Background to Lipoprotein(a) Measurement for Clinical Purposes

Lipoprotein(a) [Lp(a)] represents one of the most promising, causal independent risk factors for a chronic disease like atherosclerotic cardiovascular disease [ASCVD] that has emerged this century. Its appropriate use hinges on well-targeted implementation. This requires understanding of the whole analytical cycle (pre-analytical, analytical and post-analytical), as well as the involvement of many stakeholders. Patients who require testing need to be aware of the importance of undertaking the test. Ordering physicians need to be cognisant of target populations and the ways in which results should be applied. Laboratory scientists should consider the subtleties and intricacies of Lp(a) measurement. Implementation requires dialogue with the diagnostic industry which carries much of the responsibility for the provision of robust, validated products with traceable standardization processes which fulfil the governmental approval and monitoring processes, thereby guaranteeing minimum standards. Preventive health experts can optimize the manner in which Lp(a) results

D. Sullivan (✉) · C. Woolnough · N. Perera
Department of Chemical Pathology, Royal Prince Alfred Hospital, Sydney Local Health District, NSW Health Pathology, University of Sydney, Sydney, NSW, Australia
e-mail: David.Sullivan1@health.nsw.gov.au

J. Ramanathan
Department of Chemical Pathology, Royal Prince Alfred Hospital, Sydney Local Health District, NSW Health Pathology, University of Sydney, Sydney, NSW, Australia

Department of Medicine, Liverpool Hospital, Sydney South West Local Health District, Liverpool, NSW, Australia

T. Badrick
Royal College of Pathologists of Australasia Quality Assurance Program, St Leonards, NSW, Australia

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_18

are applied, whilst healthcare system administrators need to be able to appreciate the health and economic benefits associated with testing, as well as the limits beyond which this may become counterproductive. All stakeholders must be involved in the establishment of these objectives for the optimum widespread clinical testing of Lp(a). Ongoing attention will need to be directed towards utilization of Lp(a) testing in the future because it is likely to evolve over time, especially during the introduction of specific forms of treatment which target Lp(a) directly.

The transition from a research biomarker to a clinically relevant laboratory risk factor requires appreciation of the expectations that apply to the clinical environment in which Lp(a) will be measured. Like research laboratories, clinical service laboratories need to maintain the highest standards of process control throughout the analytical cycle. This extends from the pre-analytical sample collection and preparation to the reporting and management of Lp(a) levels. It represents a setting in which fastidious attention to correct patient identification and curation of the sample is paramount. One of the advantages of laboratory automation is the reduction in the opportunity for sources of human error such as transcription errors. Optimization and monitoring of accuracy and precision, which are components of total laboratory error, are implicit in the objectives of any analytical laboratory. Whilst research laboratories appropriately exploit a degree of independence in their approach to analytical problems, clinical service laboratories need to function within the context of laboratory networks and the wider healthcare system. This requires a high level of collaboration and collegiate activity. Such collaboration forms the basis for national and international standardization programmes, harmonization initiatives, reference range and laboratory report consensus as well as public health recommendations. More subtle considerations such as equity, accessibility and intrinsic value within the healthcare system also require careful consideration.

The clinical setting in which Lp(a) is tested also affects interpretation. Lp(a) is an acute phase reactant, so sample collection should be postponed until patients have recovered from acute inflammatory episodes or concurrent illness. One exception is in the setting of acute coronary syndrome because the opportunity to identify high-risk patients outweighs the risk of false-positive results. On the other hand, requirements for urgent laboratory turnaround time are less applicable to Lp(a) for the time being. In comparison to other analytes, technical aspects of Lp(a) measurement have posed substantially greater challenges to the implementation of Lp(a) testing than is usually the case. Nevertheless, clinicians can be assured that Lp(a) testing is fit for purpose. Furthermore, current developments are rapidly overcoming the remaining challenges, as will be discussed in more detail.

Method Selection

Before discussing Lp(a) method selection in detail, it is worth acknowledging the limited distribution of its hallmark apolipoprotein, apolipoprotein(a) [apo(a)] in nature. The presence of apo(a) in hedgehogs is thought to represent convergent

evolution (Utermann 1999), whilst its absence from other species except humans and higher apes remains difficult to explain (Utermann 1999). The absence of apo(a) in research models such as mice and rabbits has created logistic limitations because studies in such models require gene expression that is limited to one or two specific isoforms in each model. Furthermore, the high degree of polymorphism of the LPA gene locus creates an unusual degree of inter-individual genotypic and phenotypic variability. This poses important demands on clinical laboratory measurement which impact the commercial production and validation of diagnostic reagents such as monoclonal antibodies.

Another noteworthy aspect of Lp(a) is its highly skewed distribution (Kronenberg and Utermann 2013), which deviates markedly from normal distribution. Statistical analysis requires transformation, such as logarithmic transformation, or the use of non-parametric statistics. Another consequence of the skewed distribution is Lp(a)'s wide analytical range. Methods need to be able to quantify levels which may be nearly tenfold higher than the upper limit of ideal methods (Stefanutti et al. 2020).

Lp(a) is a polymorphic particle that consists of a low-density lipoprotein (LDL) particle covalently linked via a di-sulphide bond to apo(a). There is one apo(a) and one apolipoprotein B [apoB] molecule per particle (Albers et al. 1996). Apo(a) is the protein product of the highly polymorphic LPA gene locus which codes for this large protein in which a variable number of plasminogen-like kringle repeats are present (Cegla et al. 2021). In plasma, Lp(a) is the major transporter of oxidized phospholipid. This may contribute towards Lp(a)'s pro-atherogenic, pro-inflammatory and pro-thrombotic properties (Scipione et al. 2015). In theory, the separate components of Lp(a) offer alternative options for quantification.

The cholesterol and other lipid components of lipoproteins are strongly implicated in the pathophysiology of ASCVD. The measurement of Lp(a)'s cholesterol component would provide a consistent frame of reference for the pathogenicity of different lipoprotein classes, including Lp(a). On the other hand, the confounding effect of triglyceride via modification by cholesterol ester transfer protein can alter the size and density of atherogenic lipoproteins. This confuses the relationship between lipoprotein cholesterol and lipoprotein number (Carr et al. 2019). There is clear evidence that the atherogenic effect of most Apo B100-containing particles is proportional to their number rather than their cholesterol content. Efforts have been made to quantify Lp(a) in terms of cholesterol content, but the methods are not robust and evidence of specific advantages over other methods is lacking. Furthermore, the concordance of Lp(a) cholesterol measurement with Lp(a) molar results has been called into question (Konerman et al. 2012).

The measurement of Lp(a)'s oxidized phospholipid content has been deduced from immunoassay quantification via antibody E06 (Tsimikas et al. 2009). This suggests that most oxidized phospholipid is transported by Lp(a). Whilst excellent correlation between oxidized phospholipid immunoassay results and measured Lp(a) levels has been demonstrated, other techniques such as lipidomic measurement by mass spectroscopy suggest that the transport of oxidized phospholipid on Lp(a) is potentially more complex (Leibundgut et al. 2013). The main advantage of quantification of Lp(a)-associated oxidized phospholipid is the quantification of a

particular potentially toxic component, but as has already been explained, other components such as cholesterol are likely to have a modifying effect. The current clinical laboratory approach to Lp(a) is that it is best measured via its unique apo(a) component and that measurement of the cholesterol or oxidized phospholipid components of Lp(a) is not warranted because they are not consistent with the need to measure Lp(a) particle number.

Lp(a) levels have been assessed in terms of mass or molar units. LPA genotype has been assessed mainly in terms of kringle IV type 2 [KIVT2] repeats. The presence of a greater number of KIVT2 repeats is associated with a relative reduction in plasma Lp(a) molar concentrations. Metabolic turnover studies suggest that the effect is mediated via Lp(a) synthesis (Chan et al. 2019). Other genetic variations further modify the relationship (Coassin et al. 2017, 2019), but overall the relationship between mass and molar assessments of Lp(a) concentration is confounded because larger molecular weight isoforms are associated with a smaller number of particles. For example, the protein composition of Lp(a) has been shown to vary between 30 and 46% by weight (Ruhaak and Cobbaert 2020). Given that mass (mg/dL) measurements are affected by all components of the Lp(a) particle, they are inherently more variable than measures of particle concentration. This has established the need to quantify Lp(a) in molar units (nmol/L) (Ruhaak and Cobbaert 2020). Unfortunately, many of the historically seminal clinical studies were conducted at a time when this relationship was less evident. As a result, quantification in terms of mass units (g/L, mg/dl) lingers as a legacy.

Based on these principles, Lp(a) should be measured by a method (e.g. immunoassay or mass spectroscopy) that is apo(a) isoform independent. This has required the introduction of appropriate standards, calibrators and calibration protocols designed to permit estimation and reporting in molar rather than mass units. The necessary processes have been pursued throughout the past two to three decades and have involved phase 1 and phase 2 standardization programmes conducted by IFCC (International Federation of Clinical Chemistry) (Tate et al. 1998, 1999), leading to a WHO/IFCC reference reagent for immunoassay (SRM 2B) (Dati et al. 2004). This has occurred in parallel with the development of mass spectroscopy methods (Cobbaert et al. 2021) including a proposed candidate reference method (Marcovina et al. 2021). Sustained efforts by dedicated clinical scientists have put in place the associated safety and quality measures which are required to maintain laboratory performance (Marcovina and Albers 2016). This will be discussed in the next section.

Table 18.1 demonstrates that these initiatives continue to penetrate the market for diagnostic Lp(a) immunoassays. As a result, isoform-specific assays which report in molar units have started to predominate. Diagnostic companies will continue to drive this process provided such a prerequisite continues to be demanded by clinicians (Wyness and Genzen 2021). The transition from mass units (mg/dL) to molar units (nmol/L) is necessary because the inverse relationship between genetically determined apo(a) KIVT2 repeats and Lp(a) particle number confounds the concept of a single standard conversion factor (Tsimikas et al. 2018). Conversion factors also vary with the assay, Lp(a) concentration and storage. Although equivalent mass levels could be identified for the molar levels designated as medical decision-making cut-offs, mass units should be phased out as soon as possible. Lp(a)-lowering treatment may require serial measurements in individual patients,

Table 18.1 Lipoprotein(a) assay methods available

Measurement system	Number of labs enrolled in RCPA[a]	Reagent	Analytical principle (IT, immunoturbidimetric; IN, immunonephelometric)	Units of reporting	Reference interval from reagent IFU	Information sources
Thermofisher Konelab 20XTi	1	Randox	IT	nmol/L[b]	<30 mg/dL <75 nmol/L[c]	Laboratory
Beckman Coulter AU5800	1	Randox	IT	nmol/L[b]	<90 nmol/L[d]	Laboratory
Roche Cobas c501/c502/c503	9	Roche[e]	IT	nmol/L[b]	<75 nmol/L[c]	RCPA, Roche, kit IFU, laboratory
Binding site Optilite	1[h]	Binding site	IT	nmol/L[b]	<75 nmol/L	Binding site
Abbott Alinity c	1	Abbott	IT	mg/dL	<30 mg/dL	RCPA, Abbott, kit insert, laboratory
Abbott Architect c4000 c8000	2	Abbott	IT	mg/dL	<30 mg/dL	RCPA, Abbott, kit insert, laboratory
Beckman Coulter Immage 800	3[f]	Beckman Coulter	IN	mg/dL	Caucasian males 5.6–33.8 mg/dL, females 5.7–31.2 mg/dL	RCPA, Beckman coulter, kit insert
Beckman Coulter AU480	1[g]	Beckman Coulter	IT	mg/dL	<30 mg/dL	Beckman Coulter
Siemens Nephelometer II	1	Siemens	IN	g/L	<0.3 g/L[i]	Siemens, kit insert
Thermofisher Konelab 30i	1	Thermofisher	IT	g/L	0.3 g/L	Thermofisher kit insert, laboratory

Data from the 2021 RCPA Special Lipids Survey
[a]Numbers are from the 2021 RCPA Special Lipids Survey (RCPAQAP Special Lipids QAP 2021)
[b]Calibrators are standardized to the WHO/IFCC international reference reagent SRM2B
[c]Reference interval quotes Framingham data
[d]RI from https://www.austinpathology.org.au/test-directory/1247
[e]Data from the RCPA Special Lipids Program end-of-cycle report 2019
[f]Two of the three labs are outside of Australia
[g]The lab using this method is in New Zealand
[h]The lab enrolled is the manufacturer, not a pathology lab
[i]Additional gender- and ethnicity-specific RIs are given in the reagent package insert

preferably using the same assay, which makes the sole use of molar unit measurements more logical and more urgent.

Immunoassay is usually the preferred method for high-throughput laboratories due to logistic requirements. On the other hand, immunoassays may struggle in comparison to the sensitive and specific results that can be achieved with dedicated mass spectroscopy methods. Sophisticated and highly informative mass spectroscopy methods have been described for the dedicated measurement of Lp(a) (Lassman et al. 2014). Whilst such methods are generally robust, they may be more difficult to align with external quality assurance programme method groups. This is an important consideration because inter laboratory bias would lead to inconsistency in the application of the cut-offs for medical decision-making.

Clinical laboratories are also able to analyse or refer samples for LPA genetic analysis. LPA genetic polymorphisms exert most of their effects via the quantitative phenotype of the associated Lp(a) level. Currently, LPA genotyping offers little in the way of additional clinical benefit beyond quantitative plasma Lp(a) levels, so there is little incentive to study the genotype separately for clinical purposes. On the other hand, LPA genotype is one of the major contributors to "polygenic" risk scores for cardiovascular disease [CVD] (Trinder et al. 2021). The separate contribution of the two LPA gene alleles is usually managed by summation which reinforces the concept of co-dominant inheritance of the plasma Lp(a) trait. Another potential application of LPA genotyping is the possibility that pharmacogenomic assessment of LPA may identify subjects who are likely to benefit from aspirin therapy for the prevention of ASCVD (Shiffman et al. 2012). If required, routine genotyping methods such as massively parallel sequencing should suffice, even though this is not ideal for detection of nucleotide repeats.

Safety and Quality

One of the main distinguishing features of clinical laboratories is the obligation to meet the highest standards for safety as well as quality. This role is often played out "behind the scenes". Clinicians may be unaware that changes in patient results for tests they order are monitored for unexplained discrepancies (so-called delta checking) and potential medical emergencies ("critical results"). Tests are established in a manner which tries to guarantee that results are available within the timeframe required for medical decision-making. Lp(a) results are unlikely to be acutely life-threatening, so "turnaround time" is consistent with the average for non-urgent immunoassays. This contrasts with urgent assays like troponin T or I, which may warrant the provision of "point-of-care" testing options. Whilst this is not necessary for Lp(a), a case can be made for the benefits associated with rapid notification of results to patients whilst in the medical care setting because this could enhance the management of complex problems such as ASCVD risk reduction. A point-of-care test for Lp(a) has been developed, but it may not fulfil all current expectations. For example, when last reviewed, it was reported in mass units.

The quality aspects of clinical laboratory service need to be seen in a "whole of health system" context. A test like Lp(a) must be performed consistently and return the same results across all laboratories. There must be harmonization of all aspects of the analytical process, and there must be a consistent interpretive framework in which the results are applied. These requirements are usually overseen by regulatory processes which provide accreditation of diagnostic services. Sample collection for Lp(a) testing resembles the routine approach for collection and processing of blood samples. Issues concerning recent illness have been discussed already. The question of fasting status arises with lipoprotein analysis, particularly in the case of triglyceride-rich lipoproteins [TRL]. Fasting is not necessary for Lp(a) measurement because it does not affect the total plasma Lp(a) level. On the other hand, reversible redistribution from its usual density fraction to $d < 1.006$ due to reversible non-covalent association with TRL has been described (Cohn et al. 1991). The process is influenced by isoform size and may need to be considered in mechanistic studies.

Standardization of pre-analytical components such as patient preparation and sample collection is underpinned by analytical protocols which apply standard operating procedures for quantification using standard calibrators, calibration protocols and internal quality control processes. The process of establishing the framework for these essential materials has been long and rigorous (Kostner et al. 1999; Kostner and Steinmetz 1997). They allow the identification of different sources of error to ensure that tests remain within analytical and clinical performance specifications. Laboratory errors are usually conceptualized as inaccuracy ("trueness" or "bias") and imprecision (variation around the "true" value). Clinical laboratories place particular emphasis on precision because this provides a narrower range of uncertainties, thereby aiding the detection of changes which cannot be attributed to laboratory error. This needs to be considered in the context of the biological variation or fluctuations reflected by intra-individual variability. Intra-individual variability in Lp(a) levels is thought to be minor; however, levels may not be quite as static (Marcovina et al. 2018a) as some clinicians imagine. One of the reasons for clinical laboratories' slight preference for precision over accuracy is that adjustment can be made to correct accuracy via calibrators or correction factors provided precision is yielding reproducible results. Accuracy also requires a reference method and a reference standard which give the highest level of trueness and precision possible. There are currently no reference methods for Lp(a), though they are being developed. There is a reference material, but not all methods currently available are traceable to this material. There is current work on an improved reference material. Nevertheless, Lp(a) results need to be interpreted in the context of designated quantitative cut-points, so accuracy of Lp(a) measurements cannot be compromised either.

The "whole of health system" integrity of the quality of laboratory results is underpinned by systems of external quality assurance (EQA). Whereas research tests may require sample exchanges with other labs to objectively monitor accuracy and other aspects, widely used tests are scrutinized by a structured process in which unknown samples are regularly circulated for analysis and the results are aggregated to reflect the performance of individual laboratories according to their peers. Results

are compared to all participants, and they are also grouped according to method, reagents, equipment, etc. (Fig. 18.1). Target values are based on participant medians and acceptable variation about the target determined using biological variation. Duplication of some samples allows assessment of precision as well as accuracy. Figure 18.1 shows an EQA result to illustrate several considerations that apply to Lp(a). Firstly, the results were reported in mass units. The programme has responded to requests to switch to molar units. Secondly, the samples must cater to several analytes. In the case of the special lipid programme, the samples are created by spiking with a lipoprotein concentrate to create a linear escalation of concentration in duplicate samples. In this case, the concentrate lacked sufficient Lp(a) to approach the lowest medical decision point. It will be logistically difficult to adjust for the complexities of Lp(a) for several reasons. Firstly, the lipoprotein concentrate may be derived from pooled samples, in which case the samples will comprise a mixture of isoforms rather than the homozygous or heterozygous pattern expected in individual patient sera. Secondly, whilst it may be possible to increase levels towards the lower medical decision points, it may be difficult to encompass higher levels. This may become important if change in Lp(a) levels becomes a treatment target. It may be commercially difficult for EQA programmes to deal with Lp(a) separately from other lipoproteins. The fact that some EQAs add TRL and high-density lipoprotein in parallel rather than in reciprocal amounts illustrates that certain lipoprotein EQA results need to be interpreted with caution (Perera et al. 2010). The standardization and harmonization initiatives which were mentioned earlier have been particularly active in this area. EQA programmes for Lp(a) have been established and analysed (Cegla et al. 2021; Cobbaert et al. 2012), but ongoing efforts will be required (Scharnagl et al. 2019).

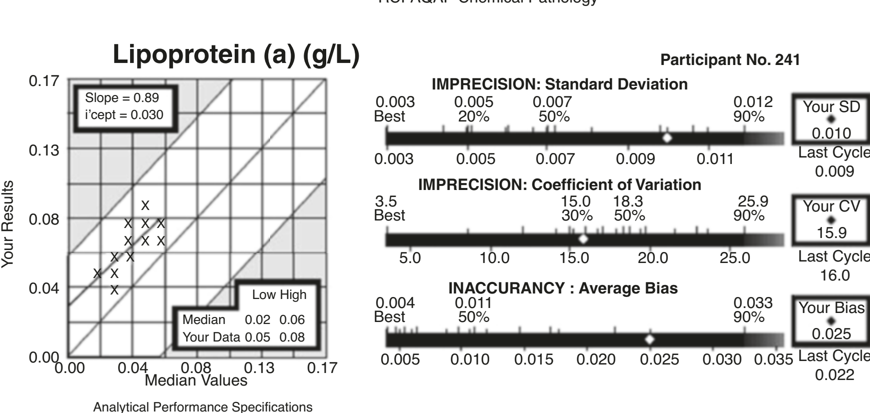

Fig. 18.1 Representative report (RCPAQAP Special Lipids QAP 2021) of EQA results for Lp(a) prior to transition to molar units

Clinical Application

Clinical laboratories need to be mindful of the clinical circumstances in which the Lp(a) test has been performed. The intra-individual variability of Lp(a) measurements before adulthood is moderate (Gidding et al. 1998). Intercurrent medical conditions and therapy may influence the result. The presence of apo(a) fragments in urine implies that Lp(a) levels may be affected by renal impairment. There is evidence to suggest a complex relationship with renal function (Kostner et al. 2000, 2001; Kronenberg et al. 1997; Cauza et al. 2003; Frank et al. 2001; Uhlig et al. 2005). Lp(a) does increase in renal impairment, but it is uncertain whether this reflects decreased catabolism. Hepatic synthesis is thought to be pivotal (Dieplinger and Utermann 1999), but hepatic function has not received a great deal of attention as a determinant of Lp(a) levels. Bile acid metabolism has a strong influence which should not be overlooked (Chennamsetty et al. 2011). Changes in endocrine status such as hypothyroidism and acromegaly can increase Lp(a) levels whilst exogenous steroid hormones can reduce Lp(a) levels. Other clinical factors affecting Lp(a) level have been summarized comprehensively (Kostner and Kostner 2004), but perhaps the most important perspective is the role of Lp(a) as an exemplar of the use of Mendelian randomization to identify a biomarker in the era of Precision Medicine (Hopewell et al. 2021).

Many patients undergoing testing for Lp(a) will be receiving therapy for dyslipidaemia, in which case those taking statins may experience an increase in Lp(a) levels (de Boer et al. 2022; Ma et al. 2019) whilst those taking evolocumab or alirocumab (Bittner et al. 2020) may experience a decrease in Lp(a) levels (O'Donoghue et al. 2019; Gencer et al. 2021). Lp(a) levels are important risk determinants in patients with familial hypercholesterolaemia due to reduced activity of LDL receptors, but the receptor pathway for Lp(a) degradation is uncertain and any increase in Lp(a) in FH remains to be fully explained (Kraft et al. 2000; Scholtz et al. 2000). Several receptors have been implicated (McCormick and Schneider 2019) in Lp(a) clearance. The functions of these receptors relate to other atherothrombotic phenomena such as inflammation and thrombosis. There is some evidence that Lp(a) may be prothrombotic (Koschinsky and Marcovina 2004) and hence a risk factor for venous thrombosis (Sofi et al. 2007) and pulmonary emboli (Ignatescu et al. 1998). This evidence is inconsistent, so Lp(a) is yet to take a place amongst the laboratory markers of thrombophilia. The remaining clinical aspects of Lp(a) have been extensively reviewed (Jawi et al. 2020; Nordestgaard and Langsted 2016; Kostner and Kostner 2017; Marcovina et al. 2018b).

Interpretation

The epidemiological and Mendelian randomization studies (Nordestgaard and Langsted 2016; Reyes-Soffer et al. 2022) which demonstrate that Lp(a) is an independent and causative risk factor for ASCVD, myocardial infarction [MI], stroke

and peripheral artery disease [PAD], as well as calcific aortic valve disease [CAVD], are based on phenotypic and genotypic techniques. Due to its skewed distribution, Lp(a) is not suitable for traditional definitions of a "normal" reference interval. Plasma Lp(a) levels are positively skewed, with the median for Caucasian populations ~20 nmol/L (<10 mg/dL). However, like many other risk factors for chronic disease, the upper limit of normal is less relevant than the threshold level at which increased risk necessitates a particular medical decision. Notions of sensitivity and specificity of Lp(a) testing will depend on the level at which such a cut-point is set. Conversely, there is the reassurance that low levels of Lp(a) are associated with reduced CVD risk (Coassin et al. 2017) and do not seem to be associated with any pathological outcomes (Langsted et al. 2021).

One perplexing aspect of Lp(a) is its variation in association with racial differences. This seems to reflect multiple genetic variations including some in the region of kringles KIV T6–T10 (Utermann 1999). Evidence suggests that this confounds the quantitative relationship between Lp(a) level and CVD risk in some racial groups (Geethanjali et al. 2003). This implies that medical decision points may need to be adjusted to take account of the widely reported effects of race on Lp(a) level (Stefanutti et al. 2020; Reyes-Soffer 2021; Ogorelkova et al. 2001). Studies are lacking in the many Indigenous groups in whom socio-economic determinants of health have created an excessive burden of CVD.

The perceived utility of Lp(a) testing depends on its ability to reclassify CVD risk, particularly amongst those who are deemed to be at "intermediate risk" by traditional methods. Lp(a) has demonstrated excellent capability in this regard in the Bruneck Study (Willeit et al. 2014). The added benefit of Lp(a) in CVD risk assessment may be presented as its contribution to the "C" statistic, but the author of studies in which this estimate has been modest or gender-specific (Cook et al. 2018; Khera et al. 2014) has cautioned against the exclusion of biomarkers on this basis (Cook 2007). Lp(a) levels may also modify the management (Burgess et al. 2018) of individuals who are not identified by routine risk factor assessments. This includes young MI patients (Berman et al. 2021) and the relatives of those with increased Lp(a). In due course, measurement of Lp(a) concentration could be considered in adults on at least one occasion to assess risk of ASCVD, but this amounts to population screening, which will require convincing cost-benefit evidence. Lp(a) testing and interpretation of results will be governed by policies determined by the local healthcare system.

Healthcare Systems

The implementation of Lp(a) testing will depend on the policies of the relevant healthcare systems. Their expectations will reflect the safety, quality and value perspectives outlined above. Clinical laboratories may consider the use of alerts and interpretive comments on laboratory reports. These may emphasize the potential need for assessment of ASCVD risk and cascade testing. Digital health technologies

and decision support systems could be employed to enhance the management of patients with elevated Lp(a), whilst telehealth services could be utilized for patients in remote areas. Calculators for ASCVD risk stratification in both primary and secondary preventions could be modified to include Lp(a) as a predictor variable, and clinical quality registries could be used to monitor the effectiveness of intervention.

Healthcare system policies will be guided by expert opinion in the form of guidelines and position statements such as those published by the Heart UK (Cegla et al. 2019), National Lipid Association (Wilson et al. 2019), Canadian Cardiovascular Society (Pearson et al. 2021), EFLM (Langlois et al. 2020), NHLBI (Marcovina et al. 2003), AHA (Grundy et al. 2019; Reyes-Soffer et al. 2022), ESC and EAS (Mach et al. 2020). An Australian perspective was recently published (Ward et al. 2019), but modification is anticipated due to the emergence of targeted therapy for Lp(a) (Swerdlow et al. 2021), the use of which will depend on its effect on CVD outcomes. A global perspective will be required, as reflected by the size of the population at risk, which exceeds one billion people (Kamstrup 2020). The previously mentioned effects of race on Lp(a) levels will necessitate policies which have been adapted to local circumstances. This includes the avoidance of financial barriers to testing, especially for those who may be at increased risk of ASCVD due to socioeconomic deprivation.

Conclusion

Cumulative research and international guidelines provide a foundation for the imminent need to manage Lp(a) in the context of diverse international healthcare systems. Evidence concerning the accuracy and application of Lp(a) measurement, the safety and efficacy of therapy and the selection and monitoring of patients for primary and secondary prevention is progressing rapidly, but it is still in its relative infancy. Laboratory measurement of Lp(a) is fit for purpose, but it requires actionable recommendations and supporting rationales along with recommendations for implementation. Furthermore, the incorporation of Lp(a) measurement into clinical practice for the prevention of ASCVD is likely to evolve via an iterative process.

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Chapter 19
Standardization of Analytical Methods for the Measurement of Lipoprotein(a): Bridging Past and Future Initiatives

Noemie Clouet-Foraison, Tomas Vaisar, and Santica M. Marcovina

Abbreviations

Apo(a)	Apolipoprotein(a)
ApoA-I	Apolipoprotein A-I
ApoB-100	Apolipoprotein B100
CV	Coefficient of variation
CVD	Cardiovascular diseases
ID	Isotope dilution
IFCC	International Federation of Clinical Chemistry
ISO	International Organization for Standardization
JCGM	Joint Committee for Guides in Metrology
KIV	Kringle IV
KIV_2	Kringle IV type 2
KIV_9	Kringle IV type 9
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LDL	Low-density lipoproteins
Lp(a)	Lipoprotein(a)
NIST	National Institute of Standards and Technology
NWRL	Northwest Lipid Research Laboratories
QC	Quality controls
SD	Standard deviation

N. Clouet-Foraison · T. Vaisar
Division of Metabolism, Endocrinology, and Nutrition, University of Washington, Seattle, WA, USA
e-mail: nclouetf@uw.edu; tvaisar@uw.edu

S. M. Marcovina (✉)
Medpace Reference Laboratories, Cincinnati, OH, USA
e-mail: s.marcovina@medpace.com

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_19

SI	International System of Units
SIL	Stable isotope labelled
WHO	World Health Organization

Standardization: What, Why, and How?

Importance of Standardization in Clinical Laboratory Medicine

Clinical laboratory measurements represent the foundation of medical care for many pathologies. Diagnostics based on laboratory test results play a major role for both clinical decision-making, treatment, and patient follow-up. It is therefore of utmost importance that clinical laboratory measurements be reliable, precise, and accurate.

From a clinical standpoint, the lack of reliability of medical test results can have significant consequences on patient care. A study by the Mayo Clinic in the United States demonstrated that a 3% error on the measurement of total cholesterol in a clinical laboratory resulted in a 10% increase of erroneous diagnostics (National Institute of Standards and Technology 2000). In the case of a false positive, patients are needlessly treated, therefore increasing the risk of iatrogenic diseases and the associated burden for medical care teams. On the other hand, in the case of a false negative, patients are not treated, which can have dramatic consequences for their life expectancy and quality of life. The lack of reliability of medical test results additionally hinders the development of new therapies and understanding of pathologies, especially in the context of clinical trials (Plebani 2006).

From a financial standpoint, erroneous diagnostics lead to multiple additional expenses for healthcare systems. The most important extra costs arise from provision of unnecessary treatments but also from the medical care of patients suffering from the consequences of a late or absent treatment. Another major source of additional cost is the repetition of medical analyses (Miller et al. 2014a). Therefore, ensuring reliability of laboratory test results represents a potentially significant financial savings. A study performed in 2000 by the National Institute of Standards and Technology (NIST) evidenced that the return on investment of the standardization of total cholesterol measurements, calculated as the ratio benefit/associated costs, was 4.5 with a social rate of return of approximately 154% (National Institute of Standards and Technology 2000). The Cholesterol Reference Method Laboratory Network also published in 2011 a study on the socioeconomic benefits associated with the standardization program for lipid measurements in clinical laboratories. The authors asked a panel of experts to estimate the share of the lipid standardization program on the reduction of death from cardiovascular disease between 1980 and 2000, assuming 24% of this reduction was due to reduction of cholesterol levels in patients. They then considered that every life-year saved represented either $50,000, $115,000, or $300,000 based on different sources (Hoerger et al. 2011). Socioeconomic benefits of implementing standardization of cholesterol in the United States resulted in estimates of $338 million dollars per year for the most

conservative model and 7.6 billion dollars per year considering medians (Hoerger et al. 2011).

Finally, in a globalized society, the availability of internationally recognized clinical decision thresholds and reference intervals for treatment is of major importance. However, this requires that clinical test performed worldwide provides comparable results, which can only be achieved through the implementation of the concept of *metrological traceability*.

Metrological Traceability, Standardization, and Harmonization

Implementing metrological traceability is the first step to standardization. The Joint Committee for Guides in Metrology (JCGM) defines metrological traceability as "the property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations, each contributing to the measurement uncertainty" (Joint Committee for Guides in Metrology (JCGM) 2012).

The first step in establishing a metrological traceability chain is to develop a primary reference measurement procedure directly connected to the units of the international system of quantities (SI). The reference method is then used to assign a value to an ultrapure material, with certified purity and associated uncertainty, called a primary reference material. This primary reference material is then used to calibrate a secondary reference measurement procedure, which is in turn used to value-assign a secondary, matrix-based reference material. In vitro diagnostic manufacturers produce their own working calibrators that are commercialized with the respective assays with value assigned by the secondary reference material. Following this chain, measurements performed in a clinical laboratory for a given biomarker are traceable directly to a unique reference point, usually called the anchor. All along the traceability chain, uncertainties associated with the measurement increase. Therefore, to obtain uncertainties that are *fit-for-clinical-purpose* at the bottom of the chain, that is, in clinical laboratories, it is necessary that uncertainties associated with the higher-order reference measurement procedure be minimized and well controlled. It is commonly considered that uncertainties associated with the reference method should be at least two-fold smaller, if not three-fold, than that expected in a clinical setting.

Establishing metrological traceability is the prime way to ensure comparability between results obtained by different methods and laboratories, independent of time and location in the world. However, there are two different possible scenarios: (1) the case of a well-known and characterized analyte for which traceability to the SI is achievable and (2) the case of a complex, heterogenous analyte for which knowledge is incomplete. In the first case, the "simplicity" of the analyte and the technical mastery of the measurement procedure make it possible to produce a high-purity primary reference material traceable to the SI, making standardization possible. However, in the second case where the analyte is of high complexity or heterogeneous, establishing standardization is hindered by methodological or technical

issues (Stoppacher et al. 2015; Josephs et al. 2019). Then, instead of proceeding with standardization, the alternative strategy is to establish harmonization of the methods by producing a matrix-based secondary reference material with value assigned by an arbitrarily designated reference method. The use of this material as common calibrator for all the other procedures usually improves comparability of the methods to a certain degree. However, since it is not anchored to the SI, harmonization of the methods does not ensure accuracy of the measurements nor stability of the values over time.

Furthermore, it is important to highlight that even though standardization and harmonization may both improve between-method comparability, they do not guarantee it (Miller et al. 2014b). Indeed, there are multiple additional pre-analytical, analytical, or post-analytical factors that can negatively impact method comparability. In particular, the use of different methodologies to isolate, target, and measure the analyte such as different antigen epitopes, different isolation techniques or detection systems and methodologies, varying interferences, and different measurement units can drastically impact method comparability. In this situation, even though standardization is achieved, method comparability will remain poor until the assays are improved, properly validated and common measurement units used. Therefore, a prerequisite for a successful standardization is that the methods demonstrate the necessary analytical performances to be deemed "standardizable."

What Does "Establishing Standardization" Mean?

International organizations and expert groups, like the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), the World Health Organization (WHO) or the International Organization for Standardization (ISO), have made major efforts in standardization and published norms, guidelines, and recommendations for the establishment of metrological traceability in clinical laboratories like the ISO 15189 for in vitro diagnostic manufacturers and the ISO 17511:2020 (International Organization for Standardization 2020). Ideally, every single biomarker or clinically relevant analyte should have its full traceability chain. In practice, this is an arduous and challenging task. The practical aspects and associated challenges will be covered in more depth in the following sections, but the first steps to establishing a metrological traceability chain for a defined analyte can be summarized as follows:

1. Production of a high-purity primary reference material. Its value should be assigned by a primary reference measurement procedure, directly traceable to the SI, and its purity should be certified.
2. Establishment of a higher-order secondary reference measurement procedure for the measurement of the concentration of the analyte in the targeted matrix. This procedure is calibrated with the pure primary reference material and should demonstrate high levels of precision and accuracy.

3. Production of a secondary reference material to disseminate traceability along the metrological chain. This matrix-based material should demonstrate a high level of commutability, that is, propensity to behave similarly to a native individual patient sample, healthy or diseased, when measured by different analytical methods.

The Specific Case of Lp(a)

Measuring Lp(a): A Major Challenge

Lp(a) is a highly complex lipoprotein formed by a particle very similar in lipid and protein composition to low-density lipoproteins (LDL) but characterized by the presence of a single molecule of a unique protein, apolipoprotein(a) [apo(a)], bound to the ApoB100 of LDL by a single disulfide bond (Schmidt et al. 2016). Circulating serum levels of Lp(a) are predominantly genetically determined by the *LPA* gene and do not substantially vary over time (Kronenberg 2016), although physiological, dietary, hormonal, and environmental factors do contribute to its biological variation (Enkhmaa et al. 2016; Garnotel et al. 1998).

Apo(a) is a heavily glycosylated protein and its presence imparts distinct properties to Lp(a) distinguishing it from LDL (Nordestgaard et al. 2010; Tsimikas 2017; Van Der Valk et al. 2016). Apo(a) shares a high amino acid sequence homology with several regions of plasminogen, including the protease domain, and the kringles IV (KIV) and V domains (Koschinsky and Marcovina 2004) and exhibits a high degree of size polymorphism. The KIV domain of apo(a) is formed by ten distinct KIV types numbered from 1 to 10. All KIV types, except KIV type 2 (KIV_2), are present in apo(a) as a single copy, while the KIV_2 varies from <3 to >40 identical repeats, resulting in the large number of apo(a) isoform sizes circulating in human plasma (Marcovina et al. 1993). Being mostly determined by its hepatic production rate, the concentration of apo(a) is largely inversely correlated to its size, smaller isoforms being produced faster (Karwatowska-Prokopczuk et al. 2021). The distribution of apo(a) serum levels and isoforms varies widely between individuals and populations of different ancestry, and because most individuals express two different alleles of the *LPA* gene, a majority of individuals presents two different size isoforms circulating in plasma (Marcovina et al. 1993; Karwatowska-Prokopczuk et al. 2021; Stefanutti et al. 2020; Kamstrup 2021; Marcovina and Albers 2016). In addition, apo(a) is heterogeneous in its glycosylation pattern, which occurs both within the core of the KIV motifs and within the linker sequences connecting the different kringles, resulting in an extremely heterogeneous population of Lp(a) particles in circulation (Marcovina and Albers 2016).

Because there is one molecule of apo(a) in Lp(a), the measurement of apo(a) is used as a surrogate measure of Lp(a) in plasma. At present, Lp(a) concentrations are reported either in nmol/L of Lp(a) protein or in mg/dL of total Lp(a) mass including

the protein, lipid, and carbohydrate components. However, because mass units rely on doubtful hypotheses regarding Lp(a) particle composition, and because the mass of apo(a) is highly variable (Marcovina and Albers 2016), guidelines now recommend the use of molar units for Lp(a) reporting in clinical laboratories (Marcovina and Albers 2016; Wilson et al. 2019; Mach et al. 2019; McCormick 2004). A variety of immunochemical methods is available to measure Lp(a) in plasma or serum such as ELISA (enzyme-linked immunosorbent assay), nephelometry, immunoturbidimetry, and fluorescent immunoassays. All of them are based on the measurement of the signal generated by the formation of a complex between apo(a) and specific monoclonal or polyclonal antibodies. By measuring the signal generated by the antigen-antibody complex in a calibration material containing a known amount of the analyte, the signal in the sample can be calculated back to a concentration of the analyte in the sample. For an assay to be accurate, (1) the antibody needs to be specific to the analyte measured, (2) the analyte should have the same structural characteristics in the sample and in the assay calibrator to ensure a similar degree of immunoreactivity per particle, (3) an appropriate reference material should be used to value-assign the assay calibrator to guarantee reproducibility and comparability of the results, and (4) harmonized protocols should be available to accurately transfer the value from the reference material to the assay calibrator and to verify that results obtained on test samples are accurate (Marcovina and Albers 2016).

Although specificity of antibodies to apo(a) is not a major issue because possible immunoreactivity with apoB-100 or plasminogen can be easily eliminated, the high degree of intra- and interindividual variation in apo(a) size, due to the variable number of KIV_2 repeats, makes it practically impossible to select assay calibrators with identical structural characteristics as individual samples. So far, only two monoclonal antibodies have been reported that bind to epitopes that are not present in KIV_2 (Marcovina and Albers 2016; Gonen et al. 2020), while all polyclonal antibodies used in various immunoassays also recognize epitopes located in the variably repeated KIV_2 domain (Marcovina and Albers 2016; Kronenberg and Tsimikas 2019). In this situation, the number of antigen-antibody complexes formed during analyses reflects the number of KIV_2 repeats and thus the isoform distribution of apo(a) in the individual rather than the apo(a) concentration. If the isoforms are smaller in the sample than in the calibrator, less immunocomplexes will be formed in the sample, resulting in underestimation of the concentration of Lp(a), and vice versa will occur in samples with larger isoforms. It has been estimated that the effect of apo(a) size variability may result in over- or underestimation of Lp(a) concentration of up to 25–30% with consequent possible misclassification of the individual's cardiovascular risk (Kamstrup 2021; Marcovina and Albers 2016; Marcovina et al. 1995).

In addition, because the concentration of apo(a) is directly correlated to its production rate which in turn is correlated to the size of apo(a), concentrations span more than 1000-fold range from <1 nmol/L in individuals with large isoforms to >1000 nmol/L in individuals with small isoforms (Kamstrup 2021; Marcovina and Albers 2016). The immunoassays must therefore have appropriate calibration dynamic ranges, meaning that most calibrators will be formed by sample pools with

high Lp(a) concentration and therefore predominantly small isoforms. Raising polyclonal antibodies that do not target the variably repeated domains of apo(a) is more challenging than it appears because of the preponderance of epitopes available in the repeated domains compared to others. Unless monoclonal antibodies are demonstrated to not cross-react with KIV_2, their use does not guarantee an isoform-size independent assay either (Kronenberg and Tsimikas 2019). At present, only one commercially available latex-enhanced turbidimetric method appears to be able to measure Lp(a) with a reduced impact from apo(a) size polymorphism (McCormick 2004). The polyclonal antibodies used in this assay are bound to latex particles, and therefore the formation of very large immunocomplexes helps reduce the impact of the size variation of apo(a). However, the unique feature of this assay is the use of five independent sample pools with Lp(a) concentrations ranging from low to high levels and pools of apo(a) isoforms ranging from predominantly large to predominantly small. This approach significantly decreases the inaccuracy of the assay associated with apo(a) size polymorphism (Marcovina and Albers 2016). However, the inverse relationship between apo(a) size and apo(a) concentration is not always consistent, and therefore the impact of apo(a) size cannot be equally minimized in all samples (Marcovina and Albers 2016; Kronenberg and Tsimikas 2019).

Overall, the high degree of size heterogeneity of apo(a), its covalent association with apoB-100, and the high sequence homology with plasminogen, parameters that all impact the analytical performances and robustness of the assays, constitute a significant challenge to the development of immunoassays to measure Lp(a) in clinical laboratories. As a consequence, there is a significant lack of comparability between methods measuring Lp(a) (Scharnagl et al. 2019; Ruhaak and Cobbaert 2020).

A History of Standardization Initiatives for Lp(a)

Following standardization efforts of apoA-I and apoB-100 (Marcovina et al. 1991), standardization initiatives for Lp(a) started in the early 1990s (Labeur et al. 1994; Albers and Marcovina 1994). Labeur and colleagues, in collaboration with the Center for Disease Control (CDC), showed for the first time the poor comparability of Lp(a) measurements performed by different clinical laboratories through two worldwide surveys organized in 1989 and 1990 (Labeur et al. 1994). Sixteen laboratories (ten in Europe and six in the USA) participated in the first survey and thirty-nine in the second (twenty-nine in Europe, seven in the USA, and three in Japan) using primarily ELISA and turbidimetric assays. For each survey, participants analyzed a set of either lyophilized or frozen samples with different Lp(a) concentrations. Depending on the methods, the interlaboratory coefficients of variation (CV) ranged from 33 to 70% highlighting that standardization efforts were urgently needed (Labeur et al. 1994).

To evaluate to what extent a common calibration material could improve comparability of Lp(a) measured by different immunoassays, Albers and Marcovina

organized a study providing collaborating laboratories with a common calibrator formed by a fresh-frozen sample with a high Lp(a) concentration and an average apo(a) isoform size along with a set of 15 samples with different Lp(a) concentrations and isoform size. Their results showed that the use of a common calibrator improved between-method comparability. However, high sample-specific variability was still observed, and results highlighted remaining issues with Lp(a) routine measurements, even across similar methods performed in different laboratories (Albers and Marcovina 1994).

Aware of the potential impact of the repeated KIV_2 on the measurement of Lp(a) by immunochemical methods, Marcovina and colleagues from the Northwest Lipid Research Laboratories (NWRL), University of Washington, produced a large number of monoclonal antibodies to specifically target apo(a). Among them, they identified and characterized one monoclonal antibody (a-40) that interacted with a unique epitope of KIV_9 and showed no interaction with KIV_2 and used it as detecting antibody to develop a sandwich ELISA assay (Marcovina et al. 1995). This method was extensively optimized and validated, and the results showed that using this ELISA assay, Lp(a) could be measured in equimolar basis, independent of the size of apo(a) in the samples. To reflect that the method accurately measures the number of apo(a) molecules and not its variable mass, Lp(a) values were reported in nmol/L (Marcovina et al. 1995).

Following these first initiatives, the IFCC created a working group for the standardization of Lp(a) assays with the aim to produce a secondary reference material to improve between-method and between-laboratory comparability (Tate et al. 1998). In a first phase, the IFCC working group organized a worldwide comparison study involving 33 diagnostic manufacturers and clinical chemistry laboratories in 12 countries performing a total of 40 different Lp(a) assays based on widely different approaches to target and detect Lp(a). The analytical performances of the 40 assay systems were evaluated by testing serum samples and manufactured Lp(a) calibrator materials for precision, linearity, and parallelism. Twenty systems were not optimized based on the use of a serum pool which tested nonlinear in sixteen systems and highly imprecise in four. When excluding the assays that did not meet the minimum acceptable analytical performances, the between-method CV was reduced down to 16% with some of the manufactured calibration materials, suggesting their potential as candidate reference materials (Tate et al. 1998).

Using results from Phase 1 regarding performances of the materials and assays, four manufactured Lp(a) materials, two lyophilized (PRM1B and PRM2B) and two liquid stabilized (PRM3B and PRM5B), were evaluated in Phase 2 in collaboration with the NWRL (Tate et al. 1999). The 4 materials were compared for analytical performance and commutability in 27 different test systems. Linearity and precision were comparable for all materials, however, depending on the material used as common calibrator; among-assay CV ranged from 11 to 22%. The material that resulted in overall best comparability between systems achieved a CV below 8% across 18 of the 27 test systems. On the basis of its analytical performances, best potential for harmonization, and documented stability, the

lyophilized serum PRM-2B was selected as a proposed secondary reference material (Tate et al. 1999).

In Phase 3 of the standardization project, the NWRL prepared and distributed 30 individual frozen plasma samples spanning a large range of Lp(a) concentrations and isoform sizes; 3 fresh-frozen quality controls with low, medium, and high Lp(a) concentrations; and the proposed reference material 2B to be tested by 22 different test systems (Marcovina et al. 2000). To be used as pure calibrator, two research laboratories isolated Lp(a) from fresh plasma from a donor with a single apo(a) isoform using two different isolation procedures. The total protein concentration of the isolated Lp(a) was determined by amino acid analysis after acid hydrolysis, and the molar concentration of apo(a) was calculated (Marcovina et al. 2000). To value-assign the proposed secondary reference material 2B, each isolate was diluted to prepare a set of standards to calibrate the apo(a)-size insensitive, double monoclonal antibody-based ELISA (Marcovina et al. 1995) designated as the reference method. Preparation 2B was analyzed six times in duplicate on three separate plates for each calibration material. The same protocol was carried out for 4 consecutive days, yielding a total of 144 values. The final value assigned to secondary material 2B was 107.1 ± 8.6 nmol/L (Marcovina et al. 2000). Using this material as common calibrator, inter-assay CV was below 10% for 18 out of the 22 measurement systems, while the others still obtained CVs above 10%. Based on the results of these international joint efforts, the proposed serum reference material SRM-2B was endorsed in 2004 by the WHO as the WHO/IFCC "*first international reference reagent for Lp(a) for immunoassays*" (Dati et al. 2004).

Overall, the results of these studies showed that the use of a suitable reference material reduced the variability related to the calibration component of the different analytical systems. However, as expected, the use of a common secondary material did not reduce the impact of the apo(a) size polymorphism, most prominent in some system than in others, resulting in strong systematic errors that impacted the overall comparability of the methods (Marcovina et al. 2000). Taken together, the results of these IFCC studies clearly highlight that not all the methods available to measure Lp(a) meet the prerequisites to be considered "standardizable."

The Current Harmonization System

Following the collaboration with the IFCC standardization working group, Marcovina and colleagues from the University of Washington developed a multistep approach to evaluate the suitability of different assays to produce comparable Lp(a) results. The first step consisted in value-assigning the assay working calibrators using the WHO/IFCC SRM-2B reference material. Six QC samples with Lp(a) concentrations ranging from low to high, and predominant apo(a) isoforms ranging from large to small, were used to validate this first calibration step. In a second step, the accuracy of Lp(a) results was verified using 80 fresh-frozen samples from individual donors selected to encompass a suitable range of Lp(a) values and apo(a)

isoforms. The QCs and the 80 donor samples were value-assigned by the ELISA-designated reference method (Marcovina et al. 1995) performing repeated measurements over several weeks and the apo(a) isoforms determined by agarose gel electrophoresis (Marcovina et al. 1993). Criteria were established to determine the acceptability of the bias obtained between the observed and the target values for these samples and the contribution of apo(a) isoform variability on the results. All requesting manufacturers received the multistep validation protocol, the WHO/IFCC reference material SRM-2B, the six QC materials, and the set of 80 samples.

Among the analytical systems evaluated during the IFCC standardization program, only one latex-enhanced turbidimetric method produced by Denka Seiken, Japan, demonstrated good agreement with Lp(a) values measured by the ELISA-designated comparison method, most of the inaccuracy being due to overestimation of Lp(a) levels in samples with large apo(a) isoform size. An extensive evaluation of the Denka Seiken method was thus performed at the NWRL and showed that careful optimization of the assay, coupled with value assignment of the five-point calibrators with the WHO/IFCC SRM-2B reference material, resulted in improved agreement of Lp(a) values with those obtained by the ELISA-designated comparison method (Marcovina and Albers 2016). Following this work, a fairly large number of manufacturers implemented the use of the Denka Seiken method on their instruments or distributed the Denka kits to be used by clinical chemistry laboratories on different analytical systems. Between 2012 and 2015, calibration and performance of 42 analytical systems based on the Denka Seiken kit using different lots of calibrators and reagents, and 6 methods from different manufacturers based on single diluted calibrators, were evaluated. The 42 analytical systems based on the use of the Denka kits meet the established performance criteria while the 6 methods using single calibrators did not, due to high apo(a) isoform-size-dependent biases. After uniform calibration with the WHO/IFCC SRM-2B reference material, the among-method CV across the 42 measurement systems on the 80 samples was 5.5% and ranged from 2.1% in samples with high Lp(a) concentrations to 10.5% in samples with low Lp(a) concentrations. These findings demonstrate that harmonization of results obtained by a variety of different instruments and different calibrator lots can be achieved in optimized test systems (Marcovina and Albers 2016).

In the period 2015–2020, the analytical performances of 29 test systems using the Denka Seiken reagents were evaluated by the NWRL. All systems were traceable to the WHO/IFCC SRM-2B through the NWRL harmonization process. As presented in Fig. 19.1a, results show that the average CV between the 29 systems was 5.0% and ranged from 2.3 to 10.5% with only one sample slightly exceeding the 10.4% desirable allowed imprecision recommended for clinical measurements by the Westgard biological variation database (Fraser 2022). Comparison of the average Lp(a) concentration calculated across the 29 systems versus the concentration measured by the Lp(a) designated comparison method shows a near perfect correlation with a 1.01 slope (Fig. 19.1b). As evidenced in Fig. 19.1c, the average relative difference from the Lp(a) assigned values was consistently below the 6.9% recommended allowable bias from the Westgard biological variation database

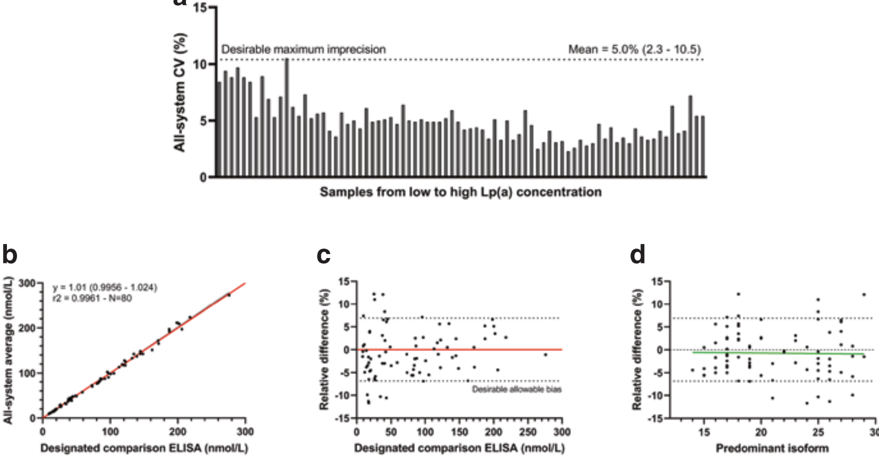

Fig. 19.1 Performance assessment of 29 systems measuring Lp(a) concentration using Denka Seiken kits harmonized to the WHO/IFCC SRM-2B. Performance was evaluated on a set of 80 individual patient samples. (**a**) Average coefficient of variation (CV) of Lp(a) values calculated across the 29 systems for the 80 samples sorted by increasing Lp(a) concentrations (nmol/L). (**b**) Average Lp(a) concentration measured by the 29 systems as a function of the Lp(a) concentrations determined by the Lp(a) designated comparison ELISA method. Black line is the unity line; slope and 95% confidence interval are indicated on the graph. (**c**) Average relative difference to the designated comparison ELISA across the 29 systems for each sample as a function of the assigned Lp(a) value measured by the designated comparison ELISA. Black dotted line is the 6.9% desirable bias recommended by the Westgard biological variation database. (**d**) Average relative difference to the designated comparison ELISA as a function of the predominant Lp(a) isoform size. Slope is not statistically different from zero (green full line)

(Fraser 2022), with the exception of 8 samples with low Lp(a) concentration. Finally, evaluation of the mean relative bias as a function of apo(a) isoforms confirmed that the Denka Seiken-based assays were minimally affected by systematic accuracy errors caused by different Lp(a) isoform sizes present in the test samples (Fig. 19.1d).

The availability of optimized assays for measuring Lp(a) with calibrators traceable to the WHO/IFCC SRM-2B reference material and with analytical performances monitored by the NWRL had a very beneficial effect on Lp(a) research: wide use and acceptance of common expression of Lp(a) values in nmol/L, comparability of data obtained in different laboratories and studies, and establishment of reliable risk thresholds for Lp(a) as a clinical biomarker of increased CVD risk (Cegla et al. 2019; Patel et al. 2021). Establishing traceability of Lp(a) measurements to the WHO/IFCC SRM-2B through the NWRL validation process provided consistent harmonization of a good number of Lp(a) assays, thus ensuring a suitable degree of comparability across traceable methods. Unfortunately, since the closure in 2020 of the NWRL by the University of Washington, the ELISA-designated comparison method and the Lp(a) harmonization protocol are no longer available, effectively ending decades of harmonization efforts.

However, in 2018, the IFCC formed a new working group for the standardization of Lp(a) assays with the intent to develop a higher-order reference measurement procedure to establish full standardization of clinical methods for measuring seven apolipoproteins including apo(a) (Ruhaak and Cobbaert 2020; Cobbaert et al. 2020). In parallel, a new method, based on the quantification of apo(a)-specific peptides by liquid chromatography-tandem mass spectrometry, has been developed and proposed as a candidate reference method for Lp(a) standardization (Marcovina et al. 2021).

Development of a New Approach to Measure Lp(a): LC-MS/MS

Targeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) is an increasingly used alternative to antibody-based immunoassays for the quantification of proteins in clinical practice (Hoofnagle et al. 2020). Although intact protein quantification has been achieved for few proteins, most methods rely on the so-called bottom-up approach where peptides liberated by proteolysis (typically with trypsin) of the target protein (proteotypic peptides) are quantified as surrogate measures of the protein by LC-MS/MS. The most common method used is Selected Reaction Monitoring, where a combination of a specific peptide precursor and associated fragment ion (a “transition”) is monitored, typically in a triple quadrupole MS to provide high selectivity and sensitivity (Kulyyassov et al. 2021). Alternatively, Parallel Reaction Monitoring performed on high-resolution instruments provides a similar degree of selectivity and sensitivity with high mass accuracy MS/MS spectra (Kulyyassov et al. 2021; Villanueva et al. 2014).

Absolute quantification of proteins by LC-MS/MS is achieved by the use of heavy stable isotope-labelled (SIL) peptides or proteins as internal standards (Villanueva et al. 2014; Shuford et al. 2017) that may be combined with external calibration. With SIL peptides, the absolute concentration of the endogenous peptides, and thereby of the target protein, is usually determined based on the absolute concentration of each individual SIL peptide. While this strategy has the advantage that peptides are easily synthesized and quantified in an SI-traceable manner, this approach however relies on the assumption that the measured endogenous protein is quantitatively (with 100% efficiency) and reproducibly digested into its proteolytic peptides. This assumption is very difficult to prove and rarely achieved because the proteolysis kinetics is highly dependent on protein structure and local environment around each proteolytic cleavage site. It also depends on stability of the formed peptides (i.e., resistance to further hydrolysis, rearrangements, and modifications).

These issues are avoided with the use of recombinant SIL full-length protein. However, producing full-length SIL proteins that are fully post-translationally modified (i.e. glycosylated) to match the endogenous proteins is generally challenging, and purification and value assignment can be highly complex. As an alternative,

multiple approaches have been investigated that entail various forms of recombinant protein fragments that include proteolytic digestion sites which produce the SIL internal peptide standards such as protein fragments or cleavable labelled peptides (Kulyyassov et al. 2021; Villanueva et al. 2014; Oeckl et al. 2018). While these are generally short and easy to characterize to the required level, these strategies are based on the assumption that digestion is comparable to that of the endogenous protein, a prerequisite for accurate absolute quantification. However, the local environment of each proteolytic cleavage site, the higher-order structure of the constructs, and their post-translational modifications differ from that of the endogenous protein which can significantly affect the proteolytic kinetics.

To achieve accurate and reproducible absolute quantification, the strategy called double isotope dilution (ID) is considered as the gold standard (Hoofnagle et al. 2020). In this approach, the SIL peptides (or protein) are spiked into the analyzed samples and into an external calibration curve constructed of the pure recombinant protein or pure proteotypic peptide (Villanueva et al. 2014; Shuford et al. 2017; Bunk and Lowenthal 2012). It was used in several higher-order reference measurement procedures for small molecules or large peptides like hemoglobin A1c and C-peptide (Hoofnagle et al. 2020) and has also been used for the absolute quantification of larger proteins (Cobbaert et al. 2020; Huynh et al. 2021; Neubert et al. 2020; Jin et al. 2019; Dittrich et al. 2018; Sabbagh et al. 2016).

Potential for Standardization Using a Higher-Order LC-MS/MS Method

Based on the characteristics described above, LC-MS/MS is also the method of choice for higher-order reference measurement procedures for the standardization of clinically relevant proteins. The LC-MS/MS method presented by Marcovina and colleagues for Lp(a) quantification laid the groundwork to this process and proposed a candidate reference measurement procedure for the quantification of apo(a) by LC-MS/MS that demonstrated high levels of accuracy and direct traceability to the SI units (Marcovina et al. 2021). This method was developed to meet the stringent requirements of a reference method for standardization and therefore focused on the absolute quantification of apo(a) rather than on multiplexing or high throughput.

To calibrate this method, the authors used a pure recombinant apo(a) that was expressed in human HEK 293 cells transfected with a 14K-pRK5 expression vector and purified by Lys-Sepharose affinity chromatography (Koschinsky et al. 1991). As previously reported, this expression protocol ensures the proper folding and glycosylation of the recombinant apo(a), which retains the same structural and functional characteristics as the endogenous protein (Koschinsky and Marcovina 2004; Koschinsky et al. 1991; Gabel and Koschinsky 1995). To provide traceability to the SI for this recombinant apo(a) calibrator, its concentration was assigned by a

higher-order reference method for amino acid quantification certified by the NIST (Lowenthal et al. 2010). The size of the recombinant apo(a) (14 kringles) was confirmed by agarose gel electrophoresis, and purity of the preparation was verified by SDS-PAGE electrophoresis (sodium dodecyl-sulfate polyacrylamide gel electrophoresis), Electrospray Differential Ion Mobility Analysis, and anion exchange Fast Protein Liquid Chromatography (Marcovina et al. 2021). This first intention assessment did not indicate the presence of significant impurities in the preparation. However, for a material to be considered as a primary reference material, its purity should be thoroughly assessed and certified, which is a significant challenge for a full-length protein (Stoppacher et al. 2015; Josephs et al. 2019). Indeed, the sheer complexity of a full-length protein in terms of post-translational modifications and glycosylation patterns, particularly for apo(a), and the considerable size of the protein (200–>800 kDa) present major obstacle to the use of most reference methods to assess purity (Stoppacher et al. 2015; Josephs et al. 2019). Consequently, even though the purity of the material reported by Marcovina and colleagues appears satisfactory, assigning a certified purity, and therefore a certified concentration, is still needed to propose this material as candidate primary reference material for apo(a) standardization.

In this published method, the authors used a double isotope dilution strategy involving both SIL peptides and the pure recombinant apo(a) for calibration (Marcovina et al. 2021) with an external six-point calibration curve constructed in a blank human serum. Each calibrator level and all samples were spiked with a mixture of the pure SIL peptides corresponding to the proteotypic peptides of interest. No sample clean-up or pre-concentration were included in this protocol, allowing to limit losses that could impact assay accuracy. The authors investigated six candidate quantification peptides of which three were validated for quantification of apo(a).

Because the method was proposed as a candidate reference method, it underwent a thorough validation. Linearity, limits of quantification, intermediate precision, reproducibility, and digestion kinetics were assessed for all measured peptides (Marcovina et al. 2021). To confirm that the recombinant apo(a) calibrator behaved similarly to endogenous apo(a), parallelism was verified, and no significant differences were found between the endogenous protein and the recombinant apo(a) calibrator. As first intent, the method was also transferred to a high-throughput LC-MS/MS in a clinical laboratory, and high degree of agreement was achieved (Marcovina et al. 2021). The authors additionally evaluated accuracy of the method on a set of 64 individual samples with a wide Lp(a) concentration range and varying isoform sizes. Comparison of Lp(a) values with those obtained by the designated ELISA method comparison (Marcovina et al. 1995) showed a Pearson correlation $r^2 = 0.958$. The Bland–Altman difference plot indicated minimal differences of LC-MS/MS values compared to ELISA with a 1.7 nmol/L mean difference (2.5%) {1.96 × SD limits of agreement −29.8 to 33.2 nmol/L} (Marcovina et al. 2021). The measurement of the WHO/IFCC SRM-2B secondary reference material produced a value of 104.7 ± 8.4 nmol/L, in close agreement with its assigned reference value of 107.1 ± 8.6 nmol/L (Dati et al. 2004). Even though this does not represent a metrologically sound assessment of the method's accuracy, it is a first indication that the

LC-MS/MS method proposed is a viable option for standardization. It further provides confidence that implementing such a method on a new preparation of a secondary reference material to replace the WHO/IFCC SRM-2B may not result in a significant change in values and risk thresholds for clinical practice.

Currently, no other LC-MS/MS method aiming at high accuracy quantification of Lp(a) has been reported. In 2018, the IFCC and the Joint Committee for Guides in Metrology (JCTLM) appointed a working group for the standardization of major apolipoproteins, including apo(a) (Cobbaert et al. 2020). It is still unclear which methodology the working group has chosen for the development and validation of the secondary reference measurement procedure for Lp(a). However, in their article from 2020, Cobbaert and colleagues laid the background and detailed the approach envisioned to finally establish traceability to the SI for Lp(a) using LC-MS/MS. The working group intends to use a peptide-based quantification approach for which the primary reference material would be a pure non-labelled proteotypic peptide of apo(a) (Cobbaert et al. 2020). As discussed earlier, this approach has the advantage that peptide purity can be more easily assessed, even though it still represents a significant challenge (Josephs et al. 2019). Synthesis and purification of peptides are well established, and reproducibility from batch to batch is more reliable than for pure recombinant proteins. However, the major downside of this approach is that peptide-based quantification is highly prone to bias, especially since, as discussed earlier, its accuracy relies on the rarely met assumption of a complete digestion of the protein, and several studies have shown that this approach can result in significant quantification errors (Shuford et al. 2017; Hoofnagle et al. 2016; Clouet-Foraison et al. 2017).

As demonstrated by Marcovina and colleagues, when a full-length recombinant apo(a) calibrator is used, it is possible to demonstrate equivalence between the calibrator and the endogenous apo(a) even when the protein digestion may not be complete. However, endorsing a pure recombinant apo(a) as a primary reference material would represent a significant challenge. Indeed, its concentration was assigned by amino acid analysis by only a single laboratory and would thus have to be confirmed by additional accredited reference laboratories to ensure its reliability. The purity of the recombinant protein will have to be certified, a process that will represent a considerable challenge (Stoppacher et al. 2015; Josephs et al. 2019). Furthermore, the stability of the protein over time will have to be established. Finally, a worldwide standardization would require that this material be made available in substantial amounts to distribute to metrology institutes for regular international comparisons and certification.

Both methodological approaches will require that the suitability of the calibration system chosen be thoroughly evaluated and validated by international comparisons between several metrology institutes and reference laboratories implementing the method in order to ensure the reliable value assignment of the primary reference material finally produced (Cobbaert et al. 2020). Its stability, homogeneity, and commutability will further have to be assessed in order to prevent accuracy errors that would later impact the entirety of the traceability chain and ultimately the patients.

LC-MS/MS for the Measurement of Lp(a) in Clinical Laboratories

LC-MS/MS measurement of Lp(a) based on quantification of apo(a) was first reported by Lassman et al. (2012, 2014). Since then, several methods to measure Lp(a) by LC-MS/MS have been reported, and they are summarized in Table 19.1 (Stefanutti et al. 2020; Ruhaak and Cobbaert 2020; Marcovina et al. 2021; Lassman et al. 2014; Blanchard et al. 2020; Van Den Broek et al. 2016). These methods include the method by Marcovina et al. (2021), proposed as a reference method, but that could also be used for routine quantification of Lp(a) after optimization for such use. Overall, all methods available, except that developed by Marcovina et al., target the same quantification peptide LFLEPTQADIALLK used by Lassman and colleagues and located in the C-terminal protease domain of apo(a). Interestingly, Marcovina et al. reported in their paper that the LFLEPTQADIALLK peptide was not selected for quantification because a larger variability of the digestion and a poorer performance in terms of repeatability and reproducibility were observed for this peptide compared to the other evaluated peptides (Marcovina et al. 2021). Lassman and Blanchard additionally included the peptide GTYSTTVTGR from the repeatable KIV-2 domain to measure the average isoform size of apo(a) (Lassman et al. 2014; Blanchard et al. 2020).

The five LC-MS/MS methods use very different calibration strategies, and only two out of the five are traceable to the WHO/IFCC SRM-2B (Stefanutti et al. 2020; Lassman et al. 2014). Even though the method developed by Marcovina and colleagues and calibrated with the primary recombinant apo(a) is not traceable to the WHO/IFCC SRM-2B, it showed a close agreement between the results obtained on the WHO/IFCC SRM-2B material and its assigned reference value (Marcovina et al. 2021). Blanchard and colleagues chose to use a double isotope dilution strategy for quantification using a stable isotope-labelled peptide and pure non-labelled analog. However, they do not report how the peptide concentrations were obtained, making it impossible to determine what reference the results are traceable to. Finally, Ruhaak and colleagues show results from the absolute quantification of apo(a) by LC-MS/MS included in a set of apolipoproteins quantified by a multiplex LC-MS/MS quantification method (Ruhaak and Cobbaert 2020; Van Den Broek et al. 2016), but neither the calibration strategy nor method validation has been reported. Precision and robustness were assessed for most methods, and intra- and inter-assay coefficients of variation were generally below 15% for all methods.

In the absence of a reference method, accuracy can be estimated by comparison of the results to a designated comparison method such as the NWRL ELISA assay in the case of Lp(a). Out of the five LC-MS/MS methods published, two have evaluated accuracy and showed excellent agreement with ELISA with minimum biases (Marcovina et al. 2021; Lassman et al. 2014). On the contrary, the other three methods only report comparison with routine immunoassays. Ruhaak and colleagues compared their method to a Roche clinical assay calibrated against the WHO/IFCC SRM-2B and showed a −16% bias with the comparison method (Ruhaak et al. 2018). Similarly, Stefanutti and colleagues reported a comparison to a routine clinical immunoassay and showed substantial deviation of the values at higher

Table 19.1 Comparison of published methods for the quantification of apo(a) by LC-MS/MS

	Lassman et al. (2014)	Van den Broek et al. (2016)	Blanchard et al. (2020)	Stefanutti et al. (2020)	Marcovina et al. (2021)
Peptide(s)	Quantification: LFLEPTQADIALLK	Quantification: LFLEPTQADIALLK confirmation: GISSTTVTGR	Quantification: LFLEPTQADIALLK	Quantification: LFLEPTQADIALLK	Quantification: TPAYYPNAGLIK, TPENYPNAGLTR, and GISSTTVTGR confirmation: LFLEPTQADIALLK
Calibration strategy	External calibration with six calibrators value assigned by the NWRL-designated comparison method for Lp(a)	Not published	Double isotope dilution strategy with synthetic non-labelled and stable isotope-labelled peptides	External calibration using the WHO/IFCC SRM-2B	Double isotope dilution with a pure recombinant apo(a) protein and stable isotope-labelled proteotypic peptides
Throughput	Medium	High	High	Medium	Low
Units	nmol/L	nmol/L	nmol/L	mg/dL	nmol/L
Traceability	WHO/IFCC SRM-2B	Not published	Not published	WHO/IFCC SRM-2B	SI-traceable
Accuracy	– Mixing experiments did not evidence dilution bias – Accuracy was estimated by comparison with the NWRL-designated comparison method for Lp(a) on a set of 80 samples. Deming slope = 0.98 (R^2 = 0.96)	– Comparison to a routine Lp(a) clinical assay from Roche validated against the NWRL and calibrated using the WHO/IFCC SRM 2B	– Comparison to an ELISA assay but results not detailed in publication	– Comparison with a routine immunoassay shows a clear deviation at higher concentrations. Spearman correlation = 0.8315	– Accuracy was estimated by comparison with the NWRL-designated comparison method for Lp(a) on a set of 64 samples. Deming slope = 0.98 (0.94–1.02)

(continued)

Table 19.1 (continued)

	Lassman et al. (2014)	Van den Broek et al. (2016)	Blanchard et al. (2020)	Stefanutti et al. (2020)	Marcovina et al. (2021)
Advantages	– Targeted quantification of Apo(a) – Intra- and inter-assay CV below 10% – Excellent comparison with the designated comparison method	– Multiplex quantification of nine apolipoproteins – Small sample volume – Short and automated sample preparation procedure – Robust reproducibility assessed over a 2-year period	– Multiplex quantification of 18 different apolipoproteins – Rapid sample preparation procedure – Intra- and inter-assay CV below 5% and 15%, respectively – Transfer to a different LC-MS/MS system showed good Bland–Altman comparison	– Targeted quantification of apo(a)	– High accuracy targeted method developed to meet the stringent requirements of a higher-order reference measurement procedure – Full validation procedure published – Intermediate precision below 10% and inter-laboratory CV below 10%
Limitations	– Long sample preparation procedure	– Validation data not published for apo(a)	– Use of an expensive commercial RapidGest kit that can be subject to lot-to-lot variations	– No data published on validation. The authors state that they used the method by Lassman and colleagues with minor modifications	– Purity of the recombinant protein calibrator was not formally assessed

concentrations, suggesting an apo(a)-size dependence of the assay (Stefanutti et al. 2020). Moreover, even though they claimed traceability to the WHO/IFCC SRM-2B material, the authors reported Lp(a) concentrations in mg/dL without specifying how these values were obtained considering that the value of SRM-2B is in nmol/L. Finally, Blanchard and colleagues did not publish comparison data but did assess transferability of the method by comparison with another LC-MS/MS procedure (Blanchard et al. 2020).

Overall, the LC-MS/MS-based quantification of apo(a) as a surrogate measure of Lp(a) concentration is a promising technique that can be used in clinical chemistry laboratories even though its overall analytical performance needs to be validated and the throughput increased for routine use. Contrary to immunoassays, LC-MS/MS can be made independent from apo(a) isoform size through the choice of a peptide outside the repeatable KIV_2 domain, and its routine implementation would allow to address the issue of isoform dependence of current clinical assays. Although LC-MS/MS requires significant initial investment in terms of instrumentation and method development, once the method is established and validated, LC-MS/MS has the potential to be a robust and powerful technique in clinical laboratories. It provides exceptional selectivity and specificity, has a potential for multiplexing, and is generally less sensitive to matrix interferences than antibody-based assays (Hoofnagle and Wener 2009; van den Broek et al. 2017).

The further development of routine LC-MS/MS assays for the measurement of Lp(a), which would meet the prerequisites to be "standardizable," could thus be envisioned as the way to achieve full standardization of Lp(a) assays. However, all the methods currently available were developed with different approaches to quantification, and different goals, and therefore, have very different performance targets and throughputs. It would nevertheless be interesting to evaluate their comparability because even though LC-MS/MS could be more easily standardizable than immunoassays, the major differences observed could influence comparability of the results.

What Is the Clinical Relevance of Standardizing Lp(a)?

So far in this book chapter, we presented the general benefits of standardization; discussed the specific issues impacting the standardization of methods to measure Lp(a); detailed the successful harmonization of Lp(a) results through the use of the WHO/IFCC secondary reference material SRM-2B that greatly improved between-method comparability, even though only for a specific group of assays; and presented the latest efforts of the IFCC to implement full traceability and standardization of Lp(a) measurements through the development of a new LC-MS/MS-based reference system. In light of all the previously discussed issues, we here propose to take a step back and ask what we consider a critical question: is Lp(a) standardization feasible and what is the clinical relevance of standardizing Lp(a) assays?

First of all, considering a metrology-oriented approach to this question, establishing full traceability and standardization is of prime importance to obtain reliable measurements. As explained earlier, standardization differs from harmonization in that it is anchored to SI units while harmonization is not (Joint Committee for Guides in Metrology (JCGM) 2012; Armbruster and Miller 2007). While harmonization allows the same degree of confidence in comparing data across methods and laboratories (Miller et al. 2014a, b), it does not prevent issues related to batch changes of the secondary reference material, nor storage or stability issues of the materials that would result in a slowly decreasing accuracy of the whole traceability chain over time. In the case of a standardized assay, the secondary reference material is traceable to a pure primary material which value is directly traceable to the SI units. This primary reference material is regularly tested within a network of higher-order primary reference measurement procedures; its purity is assessed regularly, and its value is readjusted if needed, ensuring proper anchoring of the whole traceability chain and maintained accuracy. When standardization is achieved, whenever a batch of the secondary reference material is close to depletion, a new one can be produced by value-assigning it using the same primary reference material, thus ensuring continuity from batch to batch. Obviously, this does not exclude stringent validation processes regarding the stability and commutability of the new batch (Miller et al. 2018). Nevertheless, this process is done on a regular basis by metrology institutes worldwide and can be achieved with reasonable efforts and financial input. On the contrary, when a secondary reference material is depleted without a primary reference material available to anchor the chain, like in the case of SRM-2B, producing a new batch is equivalent to starting the whole process from the beginning. For Lp(a), this means preparing the pure isolated Lp(a) fractions again, preparing several batches of candidate secondary reference materials, and repeating all the phases of the standardization efforts initiated by the IFCC working group in the 1990s, with the risk of seeing a significant change in Lp(a) values and associated clinical thresholds. This would represent tremendous work and would be a very lengthy, complex, and undesirable process. Therefore, establishing full traceability to the SI and standardization should always be the aim.

However, Lp(a) is a peculiar case. Even though the implementation of the WHO/IFCC SRM-2B efficiently improved comparability between a specific group of methods (Marcovina and Albers 2016; Kostner et al. 2018; Watts and Boffa 2018; Kronenberg 2019), clinical assays to measure Lp(a) still suffer from the exact same issues evidenced during the IFCC's first standardization process. As clearly pointed out in 2000 by Marcovina and colleagues (Marcovina et al. 2000), while the availability of a secondary reference material plays an important role in the standardization process, no reference material can eliminate the substantial differences in Lp(a) values measured by different analytical methods affected by apo(a) isoform-size heterogeneity. Similarly, recent studies reported yet again a lack of comparability across routine clinical assays harmonized to the WHO/IFCC SRM-2B (Scharnagl et al. 2019; Ruhaak and Cobbaert 2020; Wyness and Genzen 2021) with the most common issue being the variable number of epitopes on the repeatable KIV_2 domain (Ruhaak and Cobbaert 2020). As long as these assays are in use in clinical practice,

the inaccuracy of Lp(a) measurement will persist, regardless of the standardization status.

In addition, the assays most affected by apo(a) isoform-size polymorphism still measure the mass of Lp(a) and report results in mg/dL. However, determining Lp(a) mass with accuracy is not possible because of its extreme heterogeneity both within and between individuals; apo(a) is present in two alleles in more than 80% of the population, and Lp(a) composition in lipids, sphingolipids, and carbohydrate is highly variable (Marcovina and Albers 2016). Nevertheless, a large number of clinical chemistry laboratories still use these assays, and it is quite unclear how the new IFCC standardization group will deal with assays that clearly do not have the possibility to express the Lp(a) values in SI units. To complicate the issue further, while the methods based on a specific five-point calibration system appear to produce comparable results and the impact of apo(a)-size variation is greatly reduced, none of these methods are able to measure Lp(a) without any impact from apo(a) isoform size. Strictly speaking, it can be argued that these methods do not accurately measure the number of Lp(a) particles, and doubts have been cast on their suitability to express Lp(a) values in nmol/L. If this is the case, then it is reasonable to ask again what assays are considered standardizable by the new IFCC group and what criteria have been established for their selection. Finally, even after almost 20 years of harmonization efforts, studies show that some commercially available assays still exhibit suboptimal performances in terms of precision and robustness (Scharnagl et al. 2019; Ruhaak and Cobbaert 2020; Wyness and Genzen 2021). Based on these overall issues, it appears evident that even though the implementation of the elegant LC-MS/MS reference measurement procedure proposed by the IFCC group to standardize the major apolipoproteins is highly desirable, its applicability to apo(a) will prove challenging for the existing methods, and it will most certainly not solve all the issues associated with Lp(a) measurements.

Following along this line of thought, the question as to why try to standardize Lp(a) methods rises again. In a context of primary prevention, most individuals, due to the lifelong stability of Lp(a) levels in individuals, do not even need to be repeatedly tested for Lp(a) concentration (Trinder et al. 2022). For clinicians who need to measure Lp(a) in patients to estimate their risk of developing CVD, or to establish a diagnostic and treatment strategy, knowing whether Lp(a) is low or high is mostly enough because Lp(a) is a biomarker that should be used as a risk range more than as a defined cutoff point (Kronenberg and Tsimikas 2019). Moreover, Lp(a) concentrations are genetically determined (Lamon-Fava et al. 1991) and do not significantly vary over time, and behavioral and environmental factors have limited impact (Garnotel et al. 1998; Clouet-Foraison et al. 2020; Reyes-Soffer et al. 2021). Therefore, most individuals will need only one measure of Lp(a) in their lifetime (Trinder et al. 2022; Marcovina and Shapiro 2022; Deconinck et al. 2022). Moreover, the only situation where accuracy is needed is around the risk threshold where an error in the measurement will have the most impact on the patient's diagnostic and potential follow-up. Devil's advocate could thus argue that accuracy is less relevant in the case of Lp(a) and that what is really needed is precision and comparability of Lp(a) assays (Kronenberg and Tsimikas 2019; Kostner et al. 2018).

Establishing a primary reference measurement procedure, a suitable primary reference material, a traceable secondary reference material, and a SI traceability is a complex and laborious task that will require several years to be completed. Still, completing the standardization project will only be the first step to a far more gargantuan task: implementing standardization in clinical practice. Following the successful model established by the Center for Disease Control for the standardization of lipids, an international network of centrally monitored reference laboratories should be created with the task to ensure accuracy of the calibration system set in place for standardization and to certify that the accuracy of the calibration does result in accurate results in patient samples. Because ensuring accuracy does not guarantee method comparability, performances of methods and laboratories measuring Lp(a) should be regularly assessed. External quality assessment schemes and regular proficiency testing of clinical laboratories and manufacturers should be established to allow implementation of corrective strategies to ensure results comparability across methods and laboratories (Cobbaert et al. 2020).

Overall, even though standardization of Lp(a) assays is a hot and open topic, the discussion on accuracy and traceability of Lp(a) measurements should not overshadow the practical aspects of everyday measurements of Lp(a). Even though the presently available analytical methods may not possess the desired spectrum of attributes required to establish full metrological traceability, priority should be given to timely achieving uniformity of Lp(a) values and its unit of expression.

Conclusions

In this chapter, we first presented and discussed the rationale for method standardization and its necessity for clinical biomarkers. We then described the challenges associated with the measurement of Lp(a), the past standardization efforts, and the subsequent implementation of a calibration protocol spearheaded by the NWRL to verify that a common calibration traceable to the WHO/IFCC reference material SRM-2B results in harmonized Lp(a) results in patient samples in selected methods. We then presented and discussed the implementation of a new higher-order reference measurement procedure for Lp(a) using LC-MS/MS and the different strategies envisioned for this new approach. Finally, we provided a critical discussion on the feasibility of Lp(a) standardization and its practical implementation in routine clinical laboratories in contrast to what we believe is more urgently needed, which is among-method comparability and reliability of Lp(a) measurements.

There are still many obstacles to overcome and several technical challenges to solve such as developing and implementing an accurate reference measurement procedure, producing in-matrix commutable serum reference materials, and implementing the whole scheme in clinical practice. The experience of the IFCC working group on the initial standardization efforts of Lp(a) highlighted the complexity of this process for Lp(a) and clearly defined the limitations of the immunochemical methods available for its measurement indicating the need to compromise between

what are the goals of standardization and what is possible to achieve with the existing methods.

Implementing a new traceability chain is a long and arduous process that will require significant international collaboration and efforts from metrology institutes and regulatory institutions, scientific and clinical communities, assay manufacturers, and routine clinical laboratories.

Meanwhile, while the new standardization procedure is being developed, a parallel activity should be implemented to continue the verification of the manufacturers' calibration process and the comparability of Lp(a) results previously performed by the NWRL, with the ultimate goal to transition from harmonization to standardization.

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Chapter 20
On the Way to a Next-Generation Lp(a) Reference Measurement System Based on Quantitative Protein Mass Spectrometry and Molar Units

Christa Cobbaert, Liesbet Deprez, and Renee Ruhaak

Abbreviations

ALTM	All-lab total mean
Apo(a)	Apolipoprotein(a)
ApoB	Apolipoprotein B
CDC	Centers for Disease Control and Prevention
CV	Coefficient of variation
cRMP	Candidate reference measurement procedure
CSP	Clinical Standardization Programs
CVD	Cardiovascular disease
DELFIA	Dissociation-enhanced lanthanide fluorescence immunoassay
ELISA	Enzyme-linked immunosorbent assay
EQA	External Quality Assessment
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
IFCC WG APO-MS	IFCC working group on apolipoproteins by mass spectrometry
IVD	In vitro diagnostic test
K-IV_2	Repetitive kringle subunit 4, type 2 in the apo(a) molecule
LDL	Low-density lipoprotein particle
Lp(a)	Lipoprotein(a) particle

C. Cobbaert (✉) · R. Ruhaak
Leiden University Medical Center, Leiden, The Netherlands
e-mail: C.M.Cobbaert@lumc.nl; L.R.Ruhaak@lumc.nl

L. Deprez
European Commission Joint Research Center (JRC), Geel, Belgium
e-mail: liesbet.deprez@ec.europa.eu

© The Author(s), under exclusive license to Springer Nature Switzerland AG 2023
K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_20

RMP	Reference measurement procedure
SI	International System of Units
WHO	World Health Organization

Introduction

Metrological Traceability of Lp(a) Test Results

In order for Lp(a) test results to be comparable in time and space across the globe, we need traceability of test results. Traceability is the ability to trace, for example, the origin of a product, the ancestors of an individual, or the absolute value of a test result. The word “traceability” comes from the Latin verb *trahere*: to draw. Traceability can refer to documentation such as sampling procedures, laboratory methods, lab processes, etc., but as in ISO/IEC 17025, we are dealing with traceability of medical test results. It is key that test results are traceable to endorsed metrological references. Metrology refers to the science of measurement. In the case of **medical test results**, we use the wording **metrological traceability**. The current VIM definition of metrological traceability is *property of a measurement result whereby the result can be related to a reference through a documented unbroken chain of calibrations each contributing to the measurement uncertainty* (JCGM 2012). Ideally, the references are international standards that mimic the measurand of interest and have assigned values expressed in the International System of Units (SI units). For temperature and many other physical quantities, for example, mass and time traceability is relatively easily established. Also, in forensic toxicology or chemistry, the working standards are substances with defined purity and solutions of pure substances. Yet, in laboratory medicine, metrological traceability of protein test results measured in body fluids within total allowable error (Westgard QC 2014; Fraser 2001; EFLM 2019) is an enormous challenge due to the huge interindividual variability of endogenous measurands and the complexity of the human body fluid matrix in which the measurands are dissolved.

Evolution in Science and Metrology

Proteins are the primary effectors in human biology systems. Hence, complete knowledge of their structure and biological function is fundamental for understanding their potential role as promising biomarkers and/or future medical tests. Proteins from a single gene can vary widely in their amino acid sequence, and posttranslational modifications also give rise to a variety of proteoforms. As it is now recognized that the variation at the protein level is functionally relevant and much larger than the variation at the genetic level, the **Human Proteoform Project** has recently been launched. This ambitious initiative aims to define the human proteome by generating a reference set of proteoforms produced from the ~20,000 genes encoded

in the human genome. The human proteome is the set of all proteoforms expressed by humans. The underlying rationale is that proteoform-level knowledge is essential to understand biological function of proteins. Unraveling the human proteome will lead to a more refined, molecular definition of human health and disease. Conventional immunoassays that measure human proteins are generally blinded to the underlying proteoforms of the protein of interest and hence do not recognize or differentiate dysfunctional from functional proteoforms. This may of course negatively impact patient management and patient outcome. Enabling technology is needed to detect proteoforms at the molecular level. The Consortium for Top-Down Proteomics that runs the Human Proteome Initiative uses such technology (Smith et al. 2021).

Clinicians and laboratory professionals should be aware that improved technology is instrumental to advance science and the science of measurement. In order to enable accurate apolipoprotein quantitation, protein methods should specifically measure the measurand intended to be measured rather than an ill-defined mixture of proteoforms with different biological functions. This implies that in this era of precision medicine, we should introduce higher-order measurement procedures that can recognize molecular forms, that is, proteoforms, of interest. To that end, triple-quadrupole mass spectrometry-based technology has built up a reputation as a higher-order reference measurement procedure for direct, immunoassay-independent protein measurement in complex mixtures.

In this chapter, the authors clarify the current *state-of-the-science* around Lp(a) measurement, with special attention to the unique structural characteristics of the highly polymorphic apolipoprotein(a) [apo(a)] in Lp(a). The apo(a) features should be considered during Lp(a) test standardization, in order to guarantee that commercial Lp(a) tests are *fit for clinical purpose* and produce accurate results within allowable limits of uncertainty. To accomplish this, a reference measurement system that produces test results traceable to the SI units is needed. The rationale for the development of a higher-order reference measurement system and for transitioning from the former WHO-IFCC immunoassay-based reference measurement system for Lp(a) into a more robust immunoassay-independent mass spectrometry-based reference measurement system and molar units is pointed out.

Current Lp(a) Measurement Procedures

Structural Properties of Lp(a) in the Continuum of apoB-Containing Lipoprotein Classes

The Lp(a) particle is the most complex and polymorphic of all serum lipoproteins. Lp(a) was discovered by Kare Berg in 1963 and is only present in humans and the hedgehog and not in other mammals. Lp(a) is an apoB100-containing LDL-like particle that is rich in cholesterol and is associated with a unique hydrophilic, highly

glycosylated second major protein, that is, the apo(a). Apo(a) is covalently attached to apoB in Lp(a), shows ~80% amino acid homology with plasminogen, and contains multiple copies (3–> 40) of plasminogen-like kringle IV type 2 (K-IV$_2$). This peculiarity of apo(a) is known as *apo(a)-size polymorphism*. Beyond apo(a)-size polymorphism, other prominent determinants of Lp(a) levels are genetic variants such as K-IV$_2$ 4925G>A and K-IV$_2$ 4733G>A (Schachtl-Riess et al. 2021). Although apo(a) has structural homology to plasminogen, it lacks fibrinolytic activity. As a consequence of its composite structure, Lp(a) can trigger prothrombotic and antifibrinolytic actions favoring clot stability as well as atherosclerosis progression via its tendency for retention in the arterial intima, with deposition of its cholesterol load at sites of plaque formation. In addition, Lp(a) can induce inflammation and calcification in the aortic leaflet valve interstitium, leading to calcific aortic valve stenosis. Recent epidemiological and genetic evidence support the proposition that elevated concentrations of Lp(a) are causally related to atherothrombotic risk and calcific aortic valve stenosis.

The concentration of Lp(a) in blood is largely determined by genetic factors and hardly influenced by diet or lifestyle conditions. After reaching adulthood, the concentration remains constant over the lifetime of an individual. Remarkably, the interindividual variation is large as blood Lp(a) levels among individuals vary up to 1000-fold. Its frequency distribution differs across populations, being skewed to the right in Caucasians and displaying a more Gaussian distribution in Blacks. Its metabolic role is after 60 years of intense research still not understood. As Lp(a) is an independent genetic risk factor which accelerates cardiovascular disease (CVD) through pro-atherogenic, pro-thrombogenic, and proinflammatory mechanisms, the downstream consequences for patients are as devastating as in the case of untreated patients with heterozygous familial hypercholesterolemia (Reyes-Soffer et al. 2022; Kronenberg and Tsimikas 2019). Consequently, the pharmaceutical industry has invested in the development of novel therapies for Lp(a) lowering that target hepatic synthesis of apo(a). These therapies are in various phases of clinical trials, and the completion of these studies will provide critical insight into the cardiovascular benefits of Lp(a) lowering.

Characteristics and Design of Conventional Lp(a) Tests

In medical laboratories, serum/plasma Lp(a) is measured by a variety of immunochemical, CE-marked, or FDA-approved methods, based on immunoturbidimetry or immunonephelometry, dissociation-enhanced lanthanide fluorescence immunoassay (DELFIA), enzyme-linked immunosorbent assay (ELISA), or other readouts. Several requirements have to be fulfilled for an Lp(a) immunoassay to produce accurate results. Firstly, the antibodies need to be specific for the analyte intended

to be measured. Secondly, the analyte being measured in the patient sample should have the same structural characteristics as the analyte in the assay calibrator(s) to achieve the same degree of immunoreactivity per Lp(a) particle. Thirdly, an accuracy-based target value should be assigned to the assay calibrator(s) using an internationally recognized reference measurement system to guarantee consistency and comparability of results. Fourthly, common protocols should be used for transferring an accurate value from the reference material to the assay calibrators. Most commercial Lp(a) tests express Lp(a) in mass units [in mg/L or mg/dL Lp(a) mass] and make assumptions and oversimplifications to that end, which all confound Lp(a) levels (Ruhaak and Cobbaert 2020). Few routine tests express Lp(a) levels in molar units (nmol/L).

Most routine Lp(a) tests with mass units use multiple calibrators that are internally prepared by the IVD-manufacturer and produce results that are not traceable to a common internationally accepted standard. The few routine Lp(a) tests with molar units were—until recently—calibrated by the WHO-IFCC Lp(a) Reference Measurement System developed at the Northwest Lipid Metabolism and Diabetes Research Laboratories (NLMDRL), Washington DC, USA (Tsimikas et al. 2018). That reference measurement procedure (RMP) is a reference ELISA that uses monoclonal K-IV_2-independent antibodies both for capturing and detecting intact Lp(a). The RMP was calibrated with a matrix-based WHO-IFCC Reference Material named SRM2B. This reference material was value-assigned based on its apo(a) protein content and expressed in nmol/L, thus reflecting the number of Lp(a) particles.

The results of a correlation study performed in 2020 (Dikaios et al. 2023) have shown that expressing Lp(a) concentrations in molar units traceable to the SRM2B largely improves the intermethod variability (Fig. 20.1). Twenty-one human serum samples were analyzed with six methods. Three of the six methods provided the results in both molar and mass concentration units (Roche, Diasys and Sentinel). For the mass units, the average coefficient of variation (CV) between the methods was 12.8% (range: 8.4–20.5%) for all six methods and 9.7% (range: 3.4–21.8%) for the methods from Roche, Diasys, and Sentinel. For the results in molar units, the intermethod CV of these same three methods was only 3.1% (range: 1.3–5.1%) (Tables 20.1 and 20.2). These results also clearly indicate that commercial assays which are based on one serially diluted calibrator will underestimate the Lp(a) concentration in serum samples with small apo(a) isoforms.

Notwithstanding the availability of SRM2B since 2003, most commercial Lp(a) assays still use mass units. Conservatism and ignorance together with the limitations of the WHO-IFCC Lp(a) Reference Measurement System have prevented worldwide implementation of Lp(a) results in molar units. The important limitation was the fact that SRM2B could not be used directly by the test manufacturer as part of their internal traceability procedures to value-assign their product calibrators.

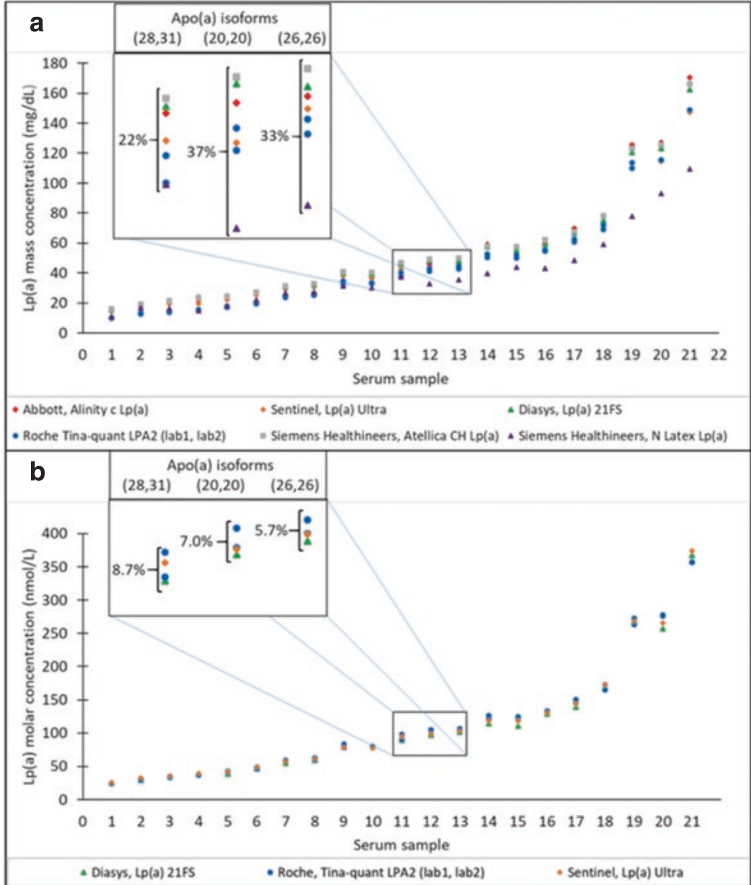


Fig. 20.1 Intermethod variability among six commercial immunoassay-based assays for the quantification Lp(a) concentration in mass (**a**) and molar units (**b**). This study was performed in 2020 (Dikaios et al. 2023). Twenty-one human serum samples were analyzed, and the average results from three replicate measurements in one run are shown in the graphs. The apo(a) isoforms present in the serum samples were determined by Western blot at the Institute of Genetic Epidemiology, Medical university of Innsbruck, Austria. All assays used a multipoint calibration curve, and for five of the six assays, the curve consisted of independent calibrator solutions. Only for the Siemens Healthineers N latex method one serially diluted calibrator was used. According to the information provided by the assay manufacturers, the values expressed in mass units were traceable to internal standards, while the values in molar units were traceable to the WHO-IFCC Reference Material SRM2B. The inserts demonstrate that at an Lp(a) **mass** level of 40–50 mg/dL, the relative difference between the highest and the lowest method result is 22% for the sample with average molecular weight apo(a) isoforms (28 and 31 K-IV) rising to 37% in a sample with small apo(a) isoforms (homozygous for 20 K-IV). In the same specimens, Lp(a) levels expressed in molar units showed an relative difference between the highest and the lowest method result ranging between 5.7 and 8.7%, that is, three to fourfold lower. The larger intermethod variability between the methods in mass units can be explained by the fact that these test results are traceable to different internal manufacturer's standards and by the fact that different calibration approaches are used, that is, five independent calibrators with specific apo(a) isoforms per calibrator level versus serial dilutions from one master calibrator solution. The variable protein/lipid content of Lp(a) particles also brings along confounded Lp(a) measurements

Table 20.1 The measurement results obtained in 2020 by measuring 21 human serum samples with 6 commercial immunoassay-based assays for the quantification Lp(a) concentration in mass units (Dikaios et al. 2023)

	Lp(a) phenotype			Lp(a) mass concentration (mg/dL)							
Serum sample	First isoform	Second isoform	Percentage first isoform (%)	Abbott Alinity c Lp(a)	Sentinel Lp(a) ultra	Siemens Healthineers Atellica CH Lp(a)	Siemens Healthineers N latex Lp(a)	Diasys Lp(a) 21FS	Roche Tina-quant LPA (lab1)	Roche Tina-quant LPA (lab2)	Intermethod CV (%)
1	19	38	5	15.1	14.1	16.1	10.9	15.8	10.2	10.1	20.5
2	32			17.9	17.1	19.3	16.7	19.4	13.3	12.7	16.1
3	32			19.9	19.6	21.4	16.6	21.8	14.7	13.9	17.5
4	22	26	15	21.9	19.6	23.3	15.0	24.2	15.6	15.4	20.5
5	27	39	90	23.2	22.1	24.3	18.2	24.9	17.9	17.1	15.6
6	26	41	95	25.6	25.7	27.2	22.1	27.1	20.3	19.4	13.6
7	33			29.5	29.7	31.3	26.9	31.1	24.8	23.9	10.6
8	29	40	70	31.0	31.2	32.9	27.3	32.3	26.3	25.1	10.6
9	30	34	60	38.2	38.4	40.9	31.4	40.1	34.8	32.6	10.2
10	25	39	85	37.4	36.4	40.3	30.3	39.3	33.5	32.7	10.2
11	28	31	60	45.2	42.2	46.8	37.6	46.0	40.7	37.7	9.1
12	20		100	46.3	42.0	49.1	32.9	48.4	43.6	41.2	12.7
13	26			47.1	45.7	50.0	35.4	48.0	44.6	43.0	10.6
14	27	34	80	59.1	51.9	58.2	39.8	57.8	52.8	50.4	12.7
15	23	35	90	53.8	52.4	57.8	44.1	55.4	52.1	50.0	8.4
16	19			59.2	55.4	62.4	43.0	59.2	55.5	54.7	11.2
17	26			69.7	60.6	67.6	48.4	67.0	62.8	60.7	11.4
18	21			78.0	70.8	78.1	59.2	75.3	72.0	68.9	9.2
19	17			125.7	110.8	123.4	77.7	120.6	109.7	113.6	14.5
20	24			127.2	114.5	125.4	93.2	123.3	115.1	115.6	9.9

(continued)

Table 20.1 (continued)

	Lp(a) phenotype			Lp(a) mass concentration (mg/dL)							
Serum sample	First isoform	Second isoform	Percentage first isoform (%)	Abbott Alinity c Lp(a)	Sentinel Lp(a) ultra	Siemens Healthineers Atellica CH Lp(a)	Siemens Healthineers N latex Lp(a)	Diasys Lp(a) 21FS	Roche Tina-quant LPA (lab1)	Roche Tina-quant LPA (lab2)	Intermethod CV (%)
21	17	30	70	170.6	147.2	166.5	109.4	162.7	148.7	148.7	13.6

Each sample was measured in three replicate measurements in one run and the average result is shown here. The apo(a) isoforms present in the serum samples were determined by Western blot at the Institute of Genetic Epidemiology, Medical university of Innsbruck, Austria. All assays used a multipoint calibration curve, and for five of the six assays, the curve consisted of independent calibrator solutions. Only for the Siemens Healthineers N latex method one serially diluted calibrator was used. According the information provided by the assay manufacturers, the values expressed in mass units were traceable to internal standards. The average intermethod CV over the 21 samples was 12.8%

Table 20.2 The measurement results obtained in 2020 by measuring 21 human serum samples with 3 commercial immunoassay-based assays for the quantification of Lp(a) concentration in molar units and the cRMP developed by the IFCC WG APO-MS (Dikaios et al. 2023)

	Lp(a) phenotype			Lp(a) molar concentration (nmol/L)						
Serum sample	First isoform	Second isoform	Percentage of first isoform (%)	Diasys Lp(a) 21FS	Roche Tina-quant LPA (lab1)	Roche Tina-quant LPA (lab2)	Sentinel Lp(a) ultra	Intermethod CV of immunoassay-based methods (%)	cRMP (LFLEPT)	Intermethod CV of all methods (%)
1	19	38	5	24.6	24.5	24.1	25.8	2.9	19.9	9.4
2	32			29.0	32.0	30.5	32.5	5.1	30.9	4.4
3	32			34.7	35.2	33.3	35.6	2.9	35.6	2.8
4	22	26	15	38.3	37.4	37.0	39.2	2.6	29.5	10.7
5	27	39	90	38.5	42.9	41.0	41.9	4.6	44.7	5.5
6	26	41	95	46.4	48.8	46.5	49.4	3.2	52.5	5.1
7	33			54.9	59.4	57.4	58.0	3.3	56.2	3.0
8	29	40	70	59.1	63.0	60.3	61.3	2.7	56.5	4.1
9	30	34	60	78.8	83.6	78.3	78.2	3.2	66.3	8.3
10	25	39	85	79.5	80.4	78.6	77.5	1.6	62.8	9.6
11	28	31	60	89.5	97.6	90.6	94.8	4.0	75.7	9.4
12	20		100	97.1	104.6	99.0	98.7	3.3	105.9	3.9
13	26			101.0	107.0	103.1	102.8	2.4	94.8	4.4
14	27	34	80	114.0	126.8	120.9	118.7	4.4	93.4	11.2
15	23	35	90	111.1	125.0	119.9	118.3	4.9	109.0	5.6
16	19			128.9	133.1	131.2	130.9	1.3	112.0	6.8
17	26			139.5	150.7	145.6	144.5	3.2	139.1	3.3
18	21			167.7	172.7	165.5	172.7	2.2	161.4	2.9
19	17			268.9	263.3	272.6	268.0	1.4	272.5	1.4
20	24			256.8	276.3	277.4	265.6	3.6	218.1	9.4
21	17	30	70	367.6	356.8	357.0	374.5	2.4	325.1	5.3

Each sample was measured in three replicate measurements in one run and the average result is shown here. The apo(a) isoforms present in the serum samples were determined by Western blot at the Institute of Genetic Epidemiology, Medical university of Innsbruck, Austria. All assays used a five-point calibration curve with independent calibrator solutions. According to the information provided by the assay manufacturers, the values in molar units were traceable to the WHO-IFCC Reference Material SRM2B. The average intermethod CV over the 21 samples was 6.0%

Effect of Lp(a) Test Inaccuracy on Clinical Utility

In the 1980s and 1990s of the twentieth century, many research groups were interested in Lp(a) studies and its implementation in patient care. Unfortunately, a turnpoint came after the publication of negative findings from the Physician Health Study in *JAMA* (*Journal of the American Medical Association*) which demonstrated no association between Lp(a) levels and the risk of future MI (myocardial infarction), stroke, or peripheral vascular disease (Ridker 1995; Ridker et al. 1993, 2001). Fortunately, Rifai et al. reanalyzed the specimens from the Physician Health Study with the gold standard reference ELISA and found that median Lp(a) in cases were significantly higher than in controls with reference ELISA, whereas no significant difference was found between cases and controls when using a commercial immunonephelometric assay (Rifai et al. 2004). It was revealed that Lp(a) test inaccuracy in the immunonephelometric assay had obscured the true relationship between Lp(a) levels and CVD in the former *JAMA* papers. It is very essential to have accurate Lp(a) tests that are not confounded by apo(a)-size polymorphism. In Fig. 20.2, the masking impact of apo(a)-size polymorphism on Lp(a) levels is visualized in case of isoform-dependent Lp(a) tests using a one-point calibration strategy (Tsimikas et al. 2018). The attenuating effect of apo(a)-size polymorphism occurs irrespective of the use of mass or molar units.

In the last years, the situation has improved as current commercially available Lp(a) tests make use of multiple independent calibrators, well spread across the Lp(a) concentration range and representing different apo(a) isoforms. This calibration strategy enables IVD manufacturers to measure Lp(a) with reduced impact of apo(a)-size polymorphism (Tsimikas et al. 2018).

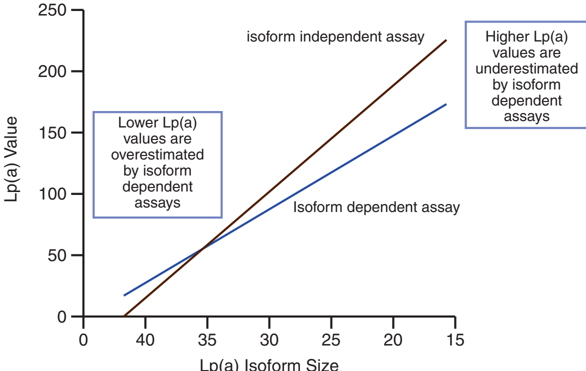


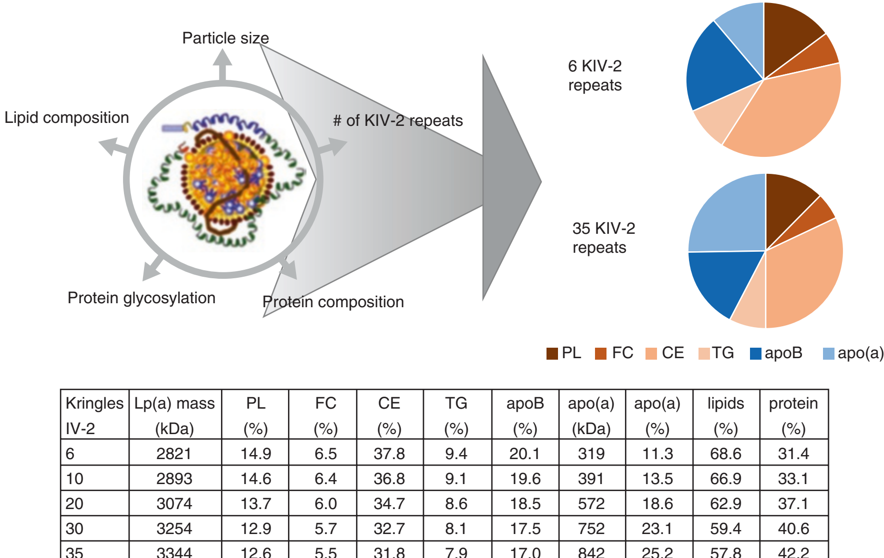
Fig. 20.2 Theoretical relationship of Lp(a) values according to isoform size in isoform-independent and isoform-dependent assays in case of a one-point calibration strategy. Lp(a) values are inversely related to isoform size, with large isoforms being associated with lower levels and vice versa. Irrespective of the expression of Lp(a) values (nmol/L or mg/dL), isoform-dependent assays will tend to overestimate low values and underestimate high values. *Lp(a)* lipoprotein(a). [Reprinted with permission from (Tsimikas et al. 2018)]

Lp(a): The Most Misunderstood Metric and the No(n)-sense *of Lp(a) Mass Results*

The determinants of variability in Lp(a) particle composition are multiple and were theoretically modeled based on existing literature (Ruhaak and Cobbaert 2020) (Fig. 20.3). To that end and beyond the effect of apo(a)-size polymorphism, post-translational modifications such as N- and O-glycosylations in apo(a) and apoB and the lipid/protein ratio were considered. Depending on the number of K-IV_2 repeats, the theoretical protein content of the Lp(a) particle varies between 30 and 46% (w/w), which inescapably confounds Lp(a) mass measurements. Based on variation in number of K-IV_2 repeats alone, the composition of lipid/protein in Lp(a) ranges from 31% (w/w) in case of apo(a) with 6 K-IV_2repeats to 42% (w/w) in case of apo(a) with 35 K-IV_2 repeats. This brings along a difference in Lp(a) mass of 19%: 2821 kDa Lp(a) mass in case of 6 K-IV_2 repeats compared to 3344 kDa Lp(a) mass in case of 35 K-IV_2 repeats. This model also clarifies why using fixed factors for converting Lp(a) particle mass into molar units that represent Lp(a) particle number—or vice versa—is metrologically not sound (Reyes-Soffer et al. 2022; Guadagno et al. 2015). Therefore, it is of utmost importance to measure Lp(a) in terms of its apo(a) component and no longer in terms of Lp(a) mass because variation in mass content can occur in each of its constituents, leading to large heterogeneity among individuals and confounded Lp(a) mass values. Because each Lp(a) particle carries one molecule of apo(a), the molar apo(a) concentration reflects unequivocally the number of Lp(a) particles.

The Degree of Lp(a) Test Harmonization and Its Impact on Cardiovascular Risk Management

In the Netherlands, the Dutch External Quality Assessment (EQA) organizer, named SKML, performs national Lp(a) surveys using frozen, native human sera. In Fig. 20.4, the national EQA data from 2018 are displayed. In total, 17 accredited laboratories participated with nearly complete data sets, which comprised 11 rounds of 2 blinded samples each. EQA samples were analyzed with two weekly intervals. Each of the blinded samples was included twice in the EQA survey, that is, in the first, respectively, and in the second half of the calendar year, to evaluate independent duplicates within a year. A scatterplot of Lp(a) mass results (in mg/L) produced by individual labs and stratified by IVD manufacturer is presented. Different IVD manufacturers are marked with specific colored symbols, whereas the all-lab total mean (ALTM) is presented with a black horizontal stripe. The overall interlaboratory variation of Lp(a) mass tests ranges from 16.4 to 32.1% at Lp(a) mass levels of ~150–450 mg/L. Siemens Healthineers demonstrates a negative bias whereas Abbott and Beckman reveal a positive bias compared to the ALTM. The Roche Lp(a) test is closest to the ALTM.



Kringles IV-2	Lp(a) mass (kDa)	PL (%)	FC (%)	CE (%)	TG (%)	apoB (%)	apo(a) (kDa)	apo(a) (%)	lipids (%)	protein (%)
6	2821	14.9	6.5	37.8	9.4	20.1	319	11.3	68.6	31.4
10	2893	14.6	6.4	36.8	9.1	19.6	391	13.5	66.9	33.1
20	3074	13.7	6.0	34.7	8.6	18.5	572	18.6	62.9	37.1
30	3254	12.9	5.7	32.7	8.1	17.5	752	23.1	59.4	40.6
35	3344	12.6	5.5	31.8	7.9	17.0	842	25.2	57.8	42.2

Fig. 20.3 Theoretical model of Lp(a) mass and compositional variation depending on apo(a) K-IV$_2$-size polymorphism based on literature. Lp(a) particle mass is dependent on the lipid/protein composition and amount, the apo(a)-size polymorphism, and the N- and O-glycosylation of apo(a) and apoB (upper left). Based on the variation in number of K-IV$_2$ repeats in apo(a) alone, the distribution of lipid/protein in Lp(a) varies from 31% (w/w) protein with 6 K-IV$_2$ repeats to 42% (w/w) protein with 35 K-IV$_2$repeats (upper right and bottom), leading to 19% difference in Lp(a) mass. *PL* phospholipids, *FC* free cholesterol, *CE* cholesteryl esters, and *TG* triglycerides. [Reprinted with permission from (Ruhaak and Cobbaert 2020)]

Taking into consideration published clinical thresholds of 150 and 300 mg/L for Lp(a) particle mass, the EQA findings suggest serious impact of Lp(a) recovery on CVD risk classification of patients and on patient management depending on the IVD manufacturers' reagents used. Yet, the systematic differences between IVD manufacturers suggest that use of a common calibrator traceable to a generally recognized point of reference (i.e., SI unit through mass spectrometry) can significantly improve the method variability. A more accurate assessment of each individual's CVD risk is also anticipated if molar Lp(a) assays rather than mass assays become the norm, because of improved reflection of low risk in patients with a small number of very large Lp(a) particles and high molecular weight apo(a) isoforms and high risk in patients with a high number of small Lp(a) particles and low molecular weight apo(a) isoforms.

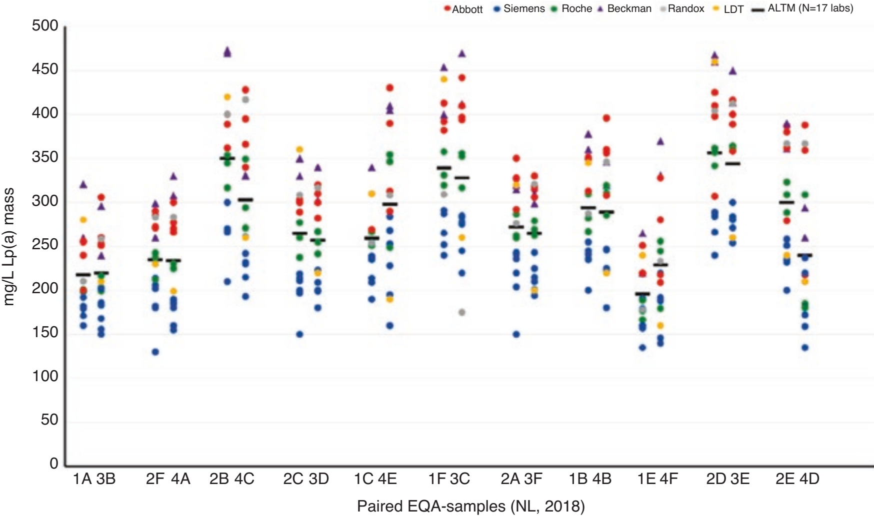


Fig. 20.4 Dutch External Quality Assessment data of Lp(a) mass as measured in 11 paired, blinded human serum samples analyzed at two weekly intervals in 2018 in 17 accredited medical laboratories. Each of the blinded samples was included twice in the EQA survey to evaluate independent duplicates within a year. For example, samples 1A and 3B are identical, but sample 1A was analyzed in the first half of 2018 and sample 3B in the second half. Absolute Lp(a) levels vary from half to double, with interlaboratory coefficients of variation ranging between 16 and 32%. [Reprinted with permission from (Ruhaak and Cobbaert 2020)]

Development of a Next-Generation SI-Traceable apo(a)/Lp(a) Reference Measurement System

A comprehensive reference measurement system is key to achieving equivalent apo(a)/Lp(a) test results that are traceable to the SI units among different measurement procedures. Essential elements of this system are an unequivocally defined analyte (including the unit) and a clearly described calibration hierarchy, which integrates the reference measurement procedure and the suitable reference materials [ISO17511:2020, (International Organization for Standardization 2020)]. Figure 20.5 shows the highest-order calibration hierarchy for Lp(a) in the envisioned Reference Measurement System.

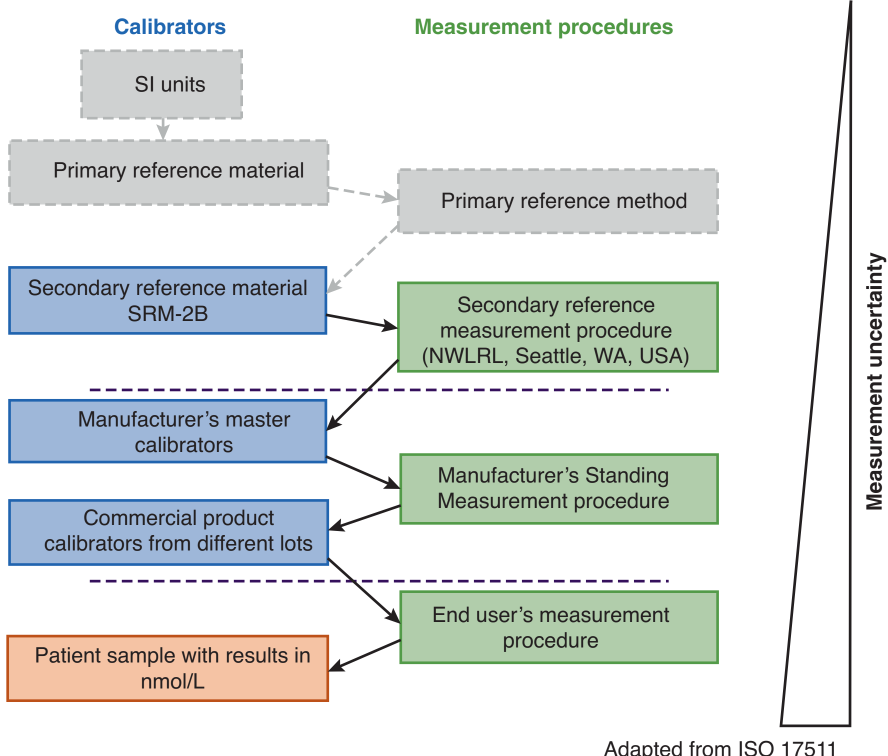


Fig. 20.5 Metrological traceability chain as outlined in ISO 17511:2021. Currently, apo(a) test results are at best traceable to WHO-IFCC secondary reference material SRM2B (blue), through an ELISA-based K-IV_2-independent method (green). However, the top of the traceability chain is not in place (grey) and under development (Cobbaert et al. 2021), as described in this chapter. To ensure SI traceability, primary reference materials (i.e., peptide-based calibrators) and a higher-order, K-IV_2-independent reference measurement procedure (MS-based cRMP) are needed. [Reprinted with permission from (Ruhaak and Cobbaert 2020)]

cRMP in a Network of Calibration Labs

A reference measurement procedure is required to transfer concentration values from primary reference materials to the secondary reference materials or to clinical specimens. Such a procedure should have very high accuracy, be specific and robust, and importantly also sustainable. Quantitative mass spectrometry proteomics, in which proteins are enzymatically digested into their representing peptides, which are then quantified, may fulfill these requirements for protein measurands. Within the IFCC WG APO-MS, a candidate reference measurement procedure (cRMP) is being developed for the quantitation of apo(a) from human serum in nmol/L, independent of the number of K-IV_2 repeats (Cobbaert et al. 2021). Notably, the measurand was specifically defined to address the number of apo(a) molecules rather than addressing the Lp(a) mass content. See Fig. 20.6.

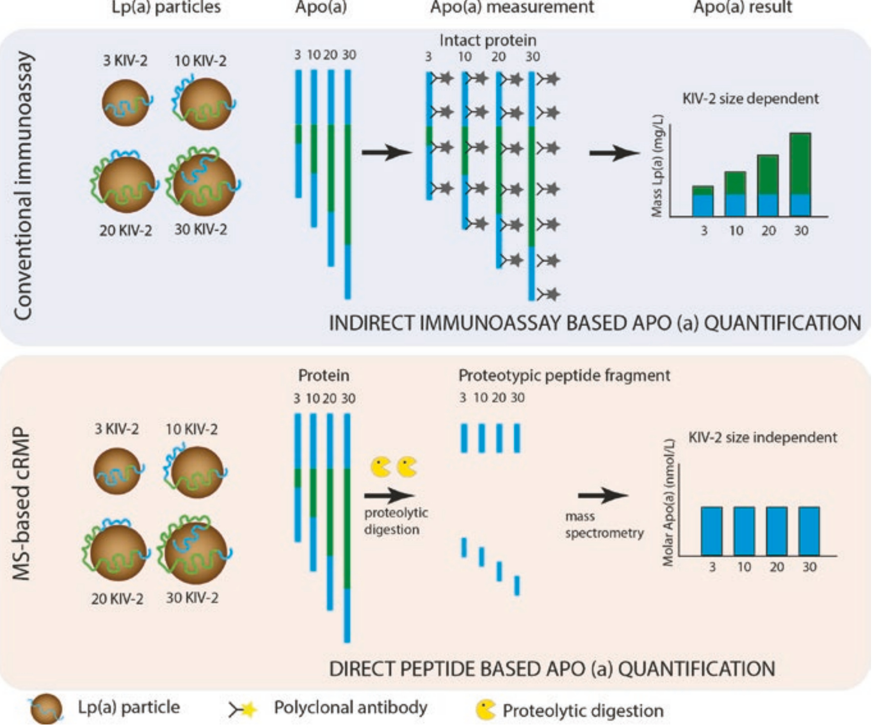


Fig. 20.6 The infographic illustrates how a high-order mass spectrometry-based measurement procedure allows for K-IV$_2$-independent measurement of apo(a) at the molecular level, in contrast to immunoassays which are by design confounded by the apo(a)-size polymorphism. Heterogeneous apo(a) with variable sizes (3, 10, 20, and 30 K-IV$_2$ repeats) are measured by immunoassay with polyclonal antibodies, resulting in a poor definition of the apo(a) measurand and K-IV$_2$-dependent results, while LC-MS-based quantitation of apo(a) at the peptide level is K-IV$_2$ independent with high specificity of the measurand and in molar concentration

A group of three laboratories allowing global coverage developed a common cRMP comprising the direct measurement of apo(a) in congruence with the six other apolipoproteins A-I, B, C-I, C-II, C-III, and E. Briefly, serum is diluted, internal standard peptides are added, and the proteins are reduced, alkylated, and digested using a combination of LysC and trypsin enzymes. Proteotypic peptides are then quantified relative to the internal standard. Importantly, MS allows not only quantitation but confirmation of identification at the same time, thus allowing definition of the measurand at the molecular level with high specificity. The developed method has an LoQ of 3.8 nmol/L, a linear range of 3.8–450 nmol/L, and a total imprecision of 9.8% (Ruhaak et al. 2023). The method inherently allows apo(a) quantitation independent of the K-IV$_2$ polymorphism through the selection of unique quantifying peptides.

Development of Primary and Secondary Reference Materials

A major requirement for establishing, implementing, and maintaining a reference system relying on an MS-based reference measurement procedure is the availability of fit-for-purpose primary and secondary reference materials. The primary reference materials will be used for the calibration of the MS-based reference measurement procedure, and they consist of peptide solutions with a well-characterized purity. A very precise determination (i.e., with very low uncertainty) of the molar concentration of these calibration solutions is essential as they will form the basis of the metrological traceability of the final results to the SI units.

The peptide-calibrated reference measurement procedure will then be used to assign the Lp(a) concentration in molar units to the secondary reference materials. These secondary reference materials should resemble the real clinical specimens measured with the immunoassay-based Lp(a) tests. The term commutability is used to express the closeness of agreement between results for a reference material and results for clinical specimens when measured with various measurement procedures (Miller et al. 2018). The complex and polymorphic nature of the Lp(a) requires the production of multiple secondary reference materials with concentrations spread across the Lp(a) concentration range and well-selected apo(a) isoforms. The IVD manufacturer will be able to use these commutable secondary reference materials directly in their internal traceability procedures to value-assign their product calibrators.

How Do Current Immunoassay-Based Lp(a) Tests Compare to the Next-Generation Reference Measurement Procedure?

The Department of Clinical Chemistry and Laboratory Medicine at the Leiden University Medical Center performs clinical Lp(a) testing using the higher-order MS-based method which recognizes each Lp(a) particle only once and allows measurement of Lp(a) in molar terms. Three-hundred sixty-five subsequent clinical samples were measured in 2020 with both the MS-based higher-order method and a Roche immunoassay (Fig. 20.7). The comparison of the immunoassay-based method (Y) and the MS-based method (X) revealed for the majority of clinical specimens a good agreement ($R > 0.975$), although in individual specimens, significant scatter was noted and sometimes extraordinary high biases occurred. The scatterplot shows a Deming regression line with a slope that does not significantly deviate from 1.0, an intercept that is not different to 0.0 and a correlation coefficient $R = 0.984$.

From the method comparison data, it can be concluded that anno 2020, the commercial Roche immunoassay test, produces **on average** equivalent results compared to the higher-order MS-based test. Both are WHO-IFCC standardized. Yet, two

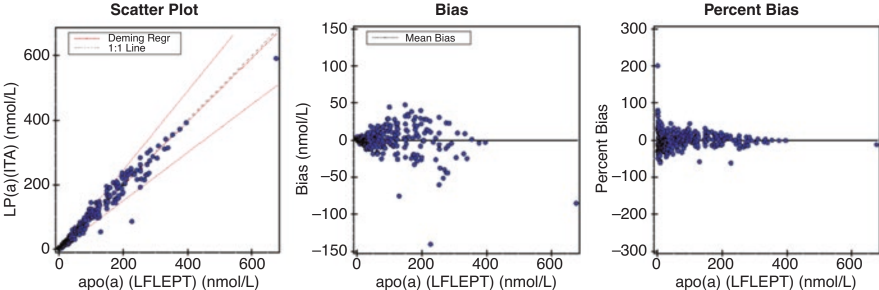


Fig. 20.7 Method comparison in molar units between Roche immunoassay and an in-house-developed immunoassay-independent higher-order MS-based method. Three-hundred sixty-five serum leftover serum specimens from the Leiden lipid clinic were compared. Deming regression line: $Y = 0.985\ X + 0.52$ in nmol/L, $X_{mean} = 75.2$ nmol/L, and $Y_{mean} = 74.6$ nmol/L; $R = 0.984$. Outliers were confirmed in independent runs. The red arrow points to a clinical specimen that demonstrates a pronounced discordance between the two assays: 227 nmol/L with the MS-based test and 87 nmol/L with the Roche immunoassay. It was demonstrated with Western blotting that apo(a) in this discordant specimen had only nine K-IV repeats (unpublished data)

observations are remarkable: substantial scatter is noted in the absolute bias plot (up to +/− 50 nmol/L) and **marked discordances may occur** in rare samples, potentially leading to discrepant classifications with effect on patient management. Apo(a) isoforms have been determined in all clinical specimens, and apo(a) isoform data corroborate the average equivalence of Lp(a) test results between MS test and IA test as well as individual scatter in clinical specimens across all apo(a) isoform groups (see Fig. 20.1). For personalized medicine, accurate Lp(a) test results are expected that are not confounded by apo(a)-size polymorphism and/or by molecular diversity arising from apo(a) variants or truncated proteins (Coassin et al. 2017). From the above head-to-head comparison, we deduce that as immunoassays are routinely used in clinical practice, immunoassay test results (Roche) are fit for clinical purpose and equivalent within total allowable error for >99% of the specimens but can be inaccurate in rare individual cases due to genetic variants/mutants who go undetected.

Transitioning from the WHO-IFCC RMS to an SI-Traceable Reference Measurement System

Background and Current WHO Reference Material for Lp(a)

The Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, USA, is the custodian laboratory for the WHO Biological Reference Material for lipoprotein(a) (i.e., IFCC SRM2B). As part of this function, it holds and distributes

this material. The current WHO/IFCC International Reference Material for lipoprotein(a) [Lp(a)] (SRM2B) was generated in 2003. Several measurement procedures for Lp(a) are traceable to this material, which was previously value-assigned through an ELISA-based K-IV_2-independent method (Tate et al. 1999). Currently, the IFCC SRM2B material is almost depleted, and also the ELISA-based K-IV_2-independent method is no longer available.

New JRC-IFCC/LNE Reference Materials

The IFCC Working Group for Apolipoproteins by Mass Spectrometry (IFCC WG APO-MS) is developing an MS-based reference measurement procedure for apolipoproteins, as well as primary and secondary reference materials for apolipoproteins including apo(a) (https://www.ifcc.org/ifcc-scientific-division/sdworking-groups/wg-apo-ms/). The MS-based reference measurement procedure will be used to assign reference values to commutable, serum-based reference materials. A reference measurement procedure and primary and secondary reference materials for Lp(a) are planned to be available in the nearby future (Cobbaert et al. 2021). The new, value-assigned, serum-based reference materials will be made available by the European Commission's Joint Research Centre (EC-JRC) (https://crm.jrc.ec.europa.eu/). The new primary peptide-based reference materials for calibration of the reference measurement procedure in IFCC-endorsed calibration labs will be stored at the Laboratoire National de Métrologie et d'Essais (LNE) (metrology@lne.fr).

Interim Solutions

The IFCC WG APO-MS members and CDC as envisioned future network coordinator of Lp(a) calibration laboratories can help IVD manufacturers, clinical trial laboratories, and EQA providers transitioning to the new IFCC reference system by making available individual donor and pooled serum materials with Lp(a) values assigned by the IFCC's LC-MS/MS method (molar units, not K-IV_2 dependent). Although this cRMP is still under development, data obtained with these materials can provide information about the agreement of a laboratory method with the MS-based method. Also, the CDC Clinical Standardization Program (CSP) will prepare a formal standardization program for guiding manufacturers on their path to molar (first step), respectively, SI-traceable (second step) Lp(a) test results and certification. In preparation of this activity, CDC-CSP will be conducting an interlaboratory comparison study for Lp(a) with routine clinical laboratories as participants. An infographic on the transition is shown at the IFCC website: https://www.ifcc.org/media/479001/210514_ifcc-apo-traceability_infographic_def.pdf.

Transitioning from Old to New Lp(a) Reference Measurement System: Recommendations to Cardiologists and Other Stakeholders

Relevance of Accurate Test Results Within Allowable Total Error

Accurate medical test results are key for safe and effective management of patients. Moreover, exchangeability of test results, reference intervals, and decision limits among healthcare institutions is essential for efficiency reasons along the patients' journey in primary, secondary, and tertiary care settings. In case of inaccuracy exceeding the total allowable error, Lp(a) test results can be misleading and harm patients due to misclassification and undetected or untreated cardiovascular risk. The (in)accuracy and analytical performance of a test affects its clinical performance as both key elements of test evaluation are interdependent. On top, flawed test results may mask the clinical utility and medical value of a test, preventing its adoption and implementation in clinical practice. These general principles also apply for Lp(a) testing. Nevertheless, a multitude of Lp(a) test design flaws are causally related to about 15 years of unjustified silence around Lp(a) testing. Currently, a revival of Lp(a) testing is taking place and its clinical relevance has been reinvented (Ellis et al. 2017).

What Were the Determinants of Inaccuracy in Case of Lp(a) Testing in the Twentieth Century?

Firstly, the definition of the measurand intended to be measured should have been unequivocal, but that was not the case. In the past decades, Lp(a) particle mass, Lp(a) particle concentration, and Lp(a) cholesterol have all been considered and used mixed. As most routine Lp(a) tests were based on an immunoassay-based readout, the apo(a)-size polymorphism confounded the Lp(a) recovery, especially in case of polyclonal antibodies. But also the type and number of calibrators are relevant: multiple calibrator levels, composed of well-selected apo(a) isoforms across the Lp(a) concentration range that mimic clinical specimens, are preferred. However, if IVD manufacturers make serial dilutions from a single calibrator, the isoform composition of the diluted calibrators is not aligned with that in the clinical specimens, and Lp(a) test results become inaccurate (Fig. 20.1). Finally, also the post-analytical phase and unit choice (mass or molar) have further aggravated the inaccuracy of Lp(a) test results due to assumptions and oversimplified models for estimating the Lp(a) particle mass (Ruhaak and Cobbaert 2020) and Figs. 20.1 and 20.4). The cumulative errors of former Lp(a) kit designs often exceeded the allowable total error, making those Lp(a) tests not *fit for clinical purpose*.

Concordance and Discordance of Contemporary Immunoassay-Based Methods Compared to a Higher-Order Mass Spectrometry-Based Method, Being a Predecessor of the SI-Traceable cRMP

Currently, immunoassay-based Lp(a) tests are still the methods of choice in medical labs. Test design and analytical performance of immunoassay-based tests have been substantially improved, especially those that are standardized against the former IFCC-WHO reference measurement system and express Lp(a) test results in molar units. In these tests, on average, equivalent results and correlation coefficients >0.975 are found between immunoassays and higher-order MS-based measurement procedures (Dikaios et al. 2023). A representative method comparison is presented in Fig. 20.7. IVD manufacturers took lessons from the past and currently use independent calibrators with well-selected apo(a) isoforms which are good mimics of clinical specimens. Notable is the fact that unexplained Lp(a) scatter—within the total allowable error zone—can be observed in the absolute/relative bias plots, also within all predominant apo(a) isoform classes when comparing routine and higher-order MS-based measurement procedures (data not shown). In addition, less than 1% discordances—exceeding the total allowable error—were found between immunoassay and higher-order MS-based measurement procedure results, pointing to further molecular variation/truncation on top of apo(a)-size polymorphism and affecting immunoassay-based measurements. As >99% of the results from current immunoassays are concordant with higher-order mass spectrometry-derived results, we can state that contemporary Lp(a) immunoassay tests are in general *fit for clinical purpose* with a manageable risk for patient harm.

Transitioning from Old to New Lp(a)/apo(a) Reference Measurement System: Recommendations for Stakeholders and End-Users

Awaiting the establishment of the complete SI-traceable Reference Measurement System for Lp(a) standardization (Cobbaert et al. 2021), it becomes obvious from Fig. 20.1 that a lot can already be gained if one starts with the movement toward **introducing molar standardization and molar units**. Therefore, a **two-step approach** is recommended as it provides IVD manufacturers, clinicians, and researchers insight in the degree of change and harmonization that can be accomplished in the transition phase. After all, average intermethod CVs from immunoassay kits expressing Lp(a) in mass units are excessive (ranging up to 21.8%) and can be reduced three to fourfold (ranging up to 5.1%) in case Lp(a) immunoassay kits are standardized in molar units and results are expressed in nmol/L apo(a). By doing so, Lp(a) tests can easily meet the total allowable error requirements, which makes

the molar Lp(a) immunoassay kits already better *fit for clinical purpose* while still being standardized to the former WHO-IFCC RMS. A second step can be made by IVD manufacturers once the SI-traceable MS-based RMS is in place and internationally recognized. To guide IVD manufacturers toward sustainable standardization of Lp(a) tests with SI traceability of Lp(a) test results, commutable, value-assigned secondary RMs will be made available by JRC which IVD manufacturers can purchase for internal standardization.

The calibration laboratories running the SI-traceable cRMP together with JRC, LNE, CDC, and the IFCC WG APO-MS will prepare the transition from old to new Lp(a) RMS along these lines, in a two-phased process.

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Chapter 21
Therapy of Elevated Lipoprotein(a)

S. Ibrahim and Erik S. G. Stroes

Expected Benefit of Lp(a) Lowering

The association between elevated lipoprotein(a) (Lp[a]) and cardiovascular risk as well as calcific aortic valve disease has been established in a large number of studies. Given the consistent genetic, mechanistic, and observational evidence supporting a (causal) role of Lp(a) in the development of different types of cardiovascular disease (CVD), the European Society of Cardiology (ESC)/European Atherosclerosis Society (EAS) guidelines recommend measuring Lp(a) levels at least once in each person's lifetime to identify individuals who have inherited an extremely elevated level (Mach et al. 2020). Consequently, this leaves clinicians facing the challenge to act on an Lp(a)-associated atherosclerotic CVD (ASCVD) risk.

Genetic Mendelian randomization studies have demonstrated that the clinical benefit of lowering Lp(a) is likely to be proportional to the absolute reduction in Lp(a) concentration (Madsen et al. 2020; Burgess et al. 2019). The first Mendelian randomization analysis, by Burgess et al. involving 80,000 patients and more than 150,000 controls, estimated that an Lp(a) reduction of 100 mg/dL (210 nmol/L) resulted in an equal ASCVD risk reduction as achieved by 1 mmol/L (38.7 mg/dL) low-density lipoprotein cholesterol (LDL-C) lowering (Burgess et al. 2019). A more recent observational study, including 58,527 participants from the Copenhagen General Population Study (CGPS), estimated that this Lp(a) equivalent is lower, approximately 55 mg/dL (116 nmol/L) (Madsen et al. 2020). Nevertheless, both studies suggest that particularly individuals with very high Lp(a) concentrations will benefit from therapies that reduce Lp(a) concentrations, which has important

S. Ibrahim · E. S. G. Stroes (✉)
Department of Vascular Medicine, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands
e-mail: s.ibrahim@amsterdamumc.nl; e.s.stroes@amsterdamumc.nl

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_21

implications for clinical practice guidelines on the use of future Lp(a) lowering therapies. These results also imply that randomized controlled trials evaluating Lp(a) lowering therapies should enroll individuals with very high baseline Lp(a) levels of 70–100 mg/dL or more, to demonstrate clinically meaningful reductions in the risk of cardiovascular events.

Since the largest ASCVD risk reduction can be achieved in patients with the highest baseline cardiovascular risk with concomitant highest Lp(a) levels, secondary prevention patients with Lp(a) elevation are the first to qualify for therapies specifically and potently lowering Lp(a). In anticipation of the new drugs, this patient group could benefit from more intensive LDL-C lowering [using either statins or proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors]. Lifestyle and blood pressure lowering are also effective in partly attenuating the Lp(a)-induced CV risk increase. Importantly, not only secondary prevention patients are likely to benefit from Lp(a) lowering. An analysis from the Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) trial demonstrated that Lp(a) was also a significant determinant of residual CVD risk in a cohort of primary prevention participants with low LDL-C (Khera et al. 2014). In this group of primary prevention patients with very high Lp(a), earlier and more intensive health behavior modification counselling and management of other ASCVD risk factors are therefore often recommended (Pearson et al. 2021).

As specific Lp(a) lowering therapies are still in development; for now, Lp(a) can be incorporated into established ASCVD risk algorithms (Nurmohamed et al. 2021a). This holds true for both primary and secondary preventions. Nurmohamed and colleagues demonstrated that in individuals with very high Lp(a) (>99th percentile), the addition of Lp(a) into ASCVD risk algorithms resulted in 31% reclassification in primary prevention and 63% reclassification in secondary prevention (Nurmohamed et al. 2021a).

Effect of Current Therapies on Lp(a)

As circulating Lp(a) levels are, for more than 85%, mediated by genetic variation at the *LPA* gene locus with only modest influence of environmental factors, pharmacological strategies to lower Lp(a) levels hold a major promise (Table 21.1) (Tsimikas and Hall 2012). New therapies potently reducing apolipoprotein(a) (apo[a]) production by the liver have shown the potential to lower Lp(a) levels by 80–98%. Nonetheless, the clinical benefit of such substantial reductions in Lp(a) remains to be established in phase 3 cardiovascular outcome trials. This leaves clinicians with existing approaches to reduce ASCVD risk, which have varying effects on Lp(a) levels.

Table 21.1 Therapeutic approaches in cardiovascular risk management that lower lipoprotein(a) concentration

Lp(a) lowering strategies	Lp(a) reduction	Cardiovascular risk reduction	FDA/ EMA approval	Reference(s)
Monoclonal PCSK9 antibodies	23.0–27.0%	Yes	Yes	Sabatine et al. (2017), Schwartz et al. (2018a), O'Donoghue et al. (2019) and Szarek et al. (2020)
PCSK9 siRNA	18.6–25.6%	Under investigation	Yes	Ray et al. (2020)
Niacin	23.0%	No	Yes	Sahebkar et al. (2016)
CETP inhibitors	10.0–56.5%	No or minimal	Not currently	Arsenault et al. (2018), Schwartz et al. (2018b), Bowman et al. (2017), Thomas et al. (2017) and Nicholls et al. (2022)
MTP inhibitors	17.0%	Not investigated	Yes	Samaha et al. (2008)
Apheresis	30.0–45.0%	Not investigated	Yes	Moriarty et al. (2019)
IL-6 inhibitors	30.0–40.0%	Under investigation	Yes	Schultz et al. (2010), McInnes et al. (2015), García-Gómez et al. (2017), Gabay et al. (2016) and Ridker et al. (2021)
Antisense oligonucleotides targeting *Apo(a)* mrna	80.0%	Under investigation	Not currently	Viney et al. (2016)
Small interfering RNA targeting *Apo(a)* mrna	>90.0%	Under investigation	Not currently	Koren et al. (2020), NCT04270760, NCT04606602

Statins

As compelling evidence has shown that statins are highly effective in reducing both LDL-C levels and cardiovascular events, this drug class now represents the cornerstone for treating patients with dyslipidemia and for attenuating cardiovascular risk in both primary and secondary preventions (Baigent et al. 2010). Clinical trials exploring the effect of statin therapy on Lp(a) levels, however, have shown mixed results. The JUPITER trial, for example, showed no median change in Lp(a) with rosuvastatin and placebo (Khera et al. 2014). Nonetheless, rosuvastatin did result in a small but statistically significant positive shift in the overall Lp(a) distribution (Khera et al. 2014). Furthermore, cell culture studies have found a time- and dose-dependent, statin-mediated increase in the expression of *LPA* mRNA and apolipoprotein(a) protein production when incubating HepG2 hepatocytes with atorvastatin (Tsimikas et al. 2020). A meta-analysis, conducted by Tsimikas et al. including 5256 patients randomized to receive rosuvastatin, atorvastatin,

pravastatin, pitavastatin, or placebo, demonstrated that Lp(a) levels increased significantly in patients starting statin therapy compared to placebo (Tsimikas et al. 2020). The mean percent change in Lp(a) level from baseline ranged from 8.5 to 19.6% in the statin groups and—0.4–2.3% in the placebo groups. A more recent meta-analysis, including 24,448 patients from 39 placebo-controlled trials with different statins, however, found no significant increases in Lp(a) levels in different types of statins, as well as different intensities of statin therapy, compared with placebo groups (de Boer et al. 2021). Although some studies suggest that statins mildly increase Lp(a) levels, in clinical practice, these effects are considered of no clinical importance since the cardiovascular benefits from statins by far outweigh the risk associated with potential mild increases in Lp(a).

Ezetimibe

Based on the available data that has been published, ezetimibe has a neutral effect on circulating Lp(a) levels. A meta-analysis including 5188 individuals from 10 randomized placebo-controlled trials demonstrated that ezetimibe therapy had no effect on plasma Lp(a) concentrations (Sahebkar et al. 2018). In addition, a subgroup analysis indicated no significant alteration in plasma Lp(a) in trials assessing the impact of ezetimibe monotherapy versus placebo nor in trials evaluating the impact of adding ezetimibe to a statin versus statin therapy alone (Sahebkar et al. 2018). Another meta-analysis, including 2337 patients with primary hypercholesterolemia from 7 randomized controlled trials, demonstrated that ezetimibe 10 mg/day significantly reduced Lp(a) by 7.1% (Awad et al. 2018). However, investigators concluded that this small reduction was not clinically significant.

PCSK9 Inhibitors

To date, PCSK9 inhibitors are the only class of LDL-C lowering drugs that has been shown to lower Lp(a) as well as reducing CVD risk. The monoclonal PCSK9 antibodies, alirocumab and evolocumab, have been evaluated in two large placebo-controlled outcome trials, together comprising more than 100,000 patient years of observation (Sabatine et al. 2017; Schwartz et al. 2018a). Both outcome trials reported a significant reduction in major adverse cardiovascular events (MACE) during treatment with PCSK9 antibodies for a median 2.3–2.7 years. Interestingly, in both trials, the absolute cardiovascular risk reduction with a PCSK9 inhibitor was higher in patients with higher baseline Lp(a) levels. In the FOURIER (Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk) study, the absolute reduction in MACE with evolocumab was 2.41% for Lp(a) >50 mg/dL versus 1.41% at lower levels (O'Donoghue et al. 2019). In the

ODYSSEY OUTCOMES (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab) trial, the absolute reduction in MACE with alirocumab was 3.7% for Lp(a) >60 mg/dL versus 0.5% in the lowest quartile for Lp(a) (Szarek et al. 2020). Since evolocumab and alirocumab also lower median baseline Lp(a) levels with 27% and 23%, respectively (Sabatine et al. 2017; Schwartz et al. 2018a), it has been a matter of debate whether and to what extent the Lp(a) lowering may contribute to the reduction in MACE, independent from the profound LDL-C reduction hallmarking these drugs. For alirocumab, it was estimated that at the 25th percentile of baseline Lp(a) (6.7 mg/dL), reduction in Lp(a) levels was small (1.6 mg/dL) and accounted for only 4% of reduction in risk of MACE, while 96% of risk reduction was attributable to lowering of corrected LDL-C (Schwartz et al. 2018a). At the 75th percentile of baseline Lp(a) (59.6 mg/dL), however, Lp(a) reduction was 13.4 mg/dL and accounted for 25% of reduction in risk of MACE.

Trials evaluating inclisiran, a small interfering RNA that suppresses PCSK9 mRNA translation and therefore reduces PCSK9 protein synthesis, have shown similar reductions in plasma levels of Lp(a) as the monoclonal antibodies (Ray et al. 2020). The ORION-10 and ORION-11 trials demonstrated Lp(a) reductions of 25.6% and 18.6%, respectively, with LDL-C reductions of approximately 50% (Ray et al. 2020). The potential benefit of inclisiran on MACE is being evaluated in the ongoing ORION-4 trial. Although PCSK9 inhibitors have been shown to reduce levels of Lp(a), with the monoclonal antibodies simultaneously reducing the risk of MACE, the extent to which Lp(a) lowering with PCSK9 inhibition contributes to the overall clinical benefit of this drug class remains, however, debatable.

Other Lipid-Modifying Agents

There are several lipid-modifying agents that have been shown to reduce Lp(a) levels in studies, however, without evidence substantiating that the Lp(a) lowering ability leads to improved clinical outcomes. Niacin, for example, which is an essential micronutrient that raises the concentration of high-density lipoprotein cholesterol (HDL-C) and reduces triglycerides (TG) as well as LDL-C concentrations, has also been shown to reduce Lp(a) levels (Sahebkar et al. 2016). A meta-analysis of 14 randomized placebo-controlled trials demonstrated a mean Lp(a) reduction of 23% in patients treated with extended-release niacin (Sahebkar et al. 2016). However, two placebo-controlled trials evaluating the cardiovascular efficacy of extended-release niacin demonstrated no cardiovascular efficacy of the drug and even showed an increased risk of serious adverse events (Boden et al. 2011; Landray et al. 2014). However, it should be noted that the median Lp(a) levels in these trials were low and the number of patients with very high Lp(a) levels was very limited, which may reduce the validity of these findings in the group with markedly elevated Lp(a) levels. Nevertheless, due to adverse effects and the lack of evidence that Lp(a) reduction with niacin is associated with a cardiovascular benefit, niacin is not recommended

in patients with Lp(a) elevation. In Europe, niacin is no longer on the market. A similar picture emerges for cholesteryl ester transfer protein (CETP) inhibitors. CETP is a liver-synthesized glycoprotein that facilitates bidirectional transfer of cholesteryl esters and triglycerides between cholesterol-rich lipoproteins (LDL, HDL) on the one hand and triglyceride-rich lipoprotein particles (VLDL) on the other hand. Inhibition of CETP has been shown to reduce levels of non-HDL cholesterol and to increase the concentration of HDL-C. Studies evaluating different CETP inhibitors have demonstrated reductions in Lp(a) ranging from 10 to 56.5% (Arsenault et al. 2018; Schwartz et al. 2018b; Bowman et al. 2017; Thomas et al. 2017; Nicholls et al. 2022). However, to date, there is no data that has related Lp(a) reductions with CETP inhibitors to clinical outcomes. In fact, three inhibitors (torcetrapib, dalcetrapib, evacetrapib) failed to show any cardiovascular benefit, and only anacetrapib showed a small cardiovascular benefit directly related to the level of non-HDLc lowering independent from Lp(a) change. The remaining CETP inhibitor in clinical trials is the obicetrapib, of which phase II trials suggest an Lp(a) reduction up to 56.5% (Nicholls et al. 2022). Awaiting the clinical outcomes trial of obicetrapib (PREVAIL; NCT03260517), none of the CETP inhibitors has been approved for therapeutic use (Nurmohamed et al. 2021b).

Plasma Lp(a) reductions can also be achieved by microsomal TG transfer protein (MTP) inhibitors. MTP is an intracellular endoplasmatic reticulum transfer protein responsible for the assembly and secretion of apolipoprotein B (apoB)-containing lipoproteins in hepatocytes and enterocytes. A study evaluating monotherapy with the MTP inhibitor lomitapide in patients with moderate hypercholesterolemia demonstrated a mean reduction in Lp(a) levels of 17% on top of an approximate 30% mean reduction in LDL-C levels (Samaha et al. 2008). However, widespread use of this drug is hampered by adverse effects, including gastrointestinal complaints, hepatic fat accumulation, and elevated liver enzymes, which occur in a significant proportion of the treated patients. Moreover, since this compound has only been approved for use in very rare patients with homozygous FH, the high price precludes its use in larger patient groups.

Other lipid-modifying agents, like bempedoic acid, fibrates, and bile acid sequestrants, have not shown any significant effect on Lp(a) levels (Rubino et al. 2021; Eraikhuemen et al. 2021).

Apheresis

Lipoprotein apheresis provides significant reductions in apolipoprotein B-containing lipoproteins including Lp(a) with median Lp(a) reductions ranging between 30 and 45% during biweekly and weekly apheresis, respectively. It is, besides PCSK9 inhibitors, the only therapeutic approach that has been shown to reduce cardiovascular events in individuals treated for elevated Lp(a), albeit no randomized trials have evaluated this therapeutic intervention (Moriarty et al. 2019). The plasmapheretic methods for reducing Lp(a) levels will be discussed extensively in another chapter.

Non-apoB-Directed Therapies

Various chronic inflammatory conditions, such as rheumatoid arthritis (RA) and Crohn's disease, have been associated with elevated Lp(a) levels (Missala et al. 2012), which is comprehensible since an IL6-responsive element is present in the promotor region of the *LPA* gene. In that regard, it is of interest to investigate whether anti-inflammatory drugs are effective in reducing Lp(a) levels and CVD risk in patients with elevated Lp(a) levels. Several clinical studies evaluating tocilizumab, a specific monoclonal antibody against interleukin 6 (IL-6), have shown reductions in plasma Lp(a) levels of approximately 30–40% in RA patients (Schultz et al. 2010; McInnes et al. 2015; García-Gómez et al. 2017; Gabay et al. 2016). Moreover, recent studies have demonstrated that IL-6 inhibition with ziltivekimab also decreases Lp(a) dose-dependently (Ridker et al. 2021). The ability of these IL-6 inhibitors to reduce CVD events is currently being evaluated in cardiovascular outcomes trials (ZEUS; NCT05021835).

Future Perspectives: Experimental Drugs

As discussed, current lipid-modifying agents only modestly reduce elevated Lp(a) levels, whereas Mendelian and epidemiological studies suggest that large reductions in Lp(a) are required in order to achieve a clinically meaningful benefit in cardiovascular outcomes. Currently, therapies targeting RNA for apo(a) have been developed in order to specifically and potently inhibit the synthesis of Lp(a). These investigational agents are currently being tested in clinical trials to determine their Lp(a) lowering ability, their safety and tolerability, and their potential to reduce CVD risk.

Antisense Oligonucleotides

Pelacarsen [formerly known as IONIS-APO(a)-LRX and AKCEA-APO(a)-LRX] is a gal-nac-conjugated, single-stranded antisense oligonucleotide that targets hepatic apo(a) mRNA. The gal-nac moiety increase selective hepatic uptake of the antisense via the asiologlycoprotein receptor 1; after hepatic uptake the antisense leads to degradation of the apo(a)-messenger RNA within the nucleus of the hepatocyte. The drug has shown median Lp(a) reductions of 80% with good tolerability of monthly injections of the GalNac antisense (Viney et al. 2016). With 8324 patients enrolled, the HORIZON cardiovascular outcomes trial is expected to report in 2025 (NCT04023552). Antisense therapy targeting Lp(a) will be further discussed in the next chapter.

Small Interfering RNA

There are currently two small interfering RNA (siRNA) agents targeting *LPA* that are being tested in different phases in clinical trials. SiRNAs are mostly 21–25 nucleotides long, double-stranded RNA that have sequence-homology-driven gene-knockdown capability (Tromp et al. 2020). Following incorporation into the RNA-induced silencing complex (RISC) in the plasma, the modified RISC allows for gene blocking by binding and then degrading the mRNA produced by the gene by the protein Argonaut-1. Two major differences with the antisense mechanism is it's mode of action in the cytoplasm rather than the nucleus; and second the long half-life of the inhibitory effect of the siRNA due to the stability of the siRNA in the RISC complex. Olpasiran (formerly known as AMG-890 and ARO-LPA) is one example of an siRNA targeting apo(a) mRNA, which has shown reductions in Lp(a) levels of more than 90% with no safety concerns identified in healthy volunteers in a phase 1 study (Koren et al. 2020). A phase 2 dose finding study was recently completed in 290 patients with established ASCVD and Lp(a) levels ≥200 nmol/L, showing a 97.4% reduction in placebo-adjusted changes of Lp(a) with the 75-mg dose administered once every 3 months sucutaneously, (O'Donoghue et al. 2022). A cardiovascular outcomes trial using Olpasiran has recently started (Ocean(a); NCT05581303). Another GalNAc-conjugated siRNA targeting the mRNA transcript of *LPA* is SLN360 has reported a >95% Lp(a) reduction in the APOLLO trial (Nissen et al. 2022). A larger phase II trial using SLN360 in patient with atherosclerotic cardiovascular disease and Lp(a) elevation is expected to start in the beginning of 2023 (NCT05537571).

Conclusion

In summary, current available therapeutic agents have only mild to moderate Lp(a) lowering effects with PCSK9 inhibitors and apheresis being the only existing therapeutic approaches that have been shown to lower Lp(a) levels and reduce CVD risk. The magnitude of treatment benefit for PCSK9 inhibitors is associated with baseline Lp(a) levels and seems to be associated with the degree of Lp(a) reduction. Specific and potent RNA-targeted interventions have the potential to greatly reduce Lp(a) concentrations. Cardiovascular outcomes trials will have to show whether such substantial Lp(a) reductions are associated with meaningful clinical benefit, the outcomes of which are expected in 2025 (HORIZON) and 2026/7 (Ocean(a)), respectively.

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Chapter 22
Antisense Oligonucleotide Therapy to Treat Elevated Lipoprotein(a)

Sotirios Tsimikas

Since the initial report of the discovery of lipoprotein(a) [Lp(a)] in a single-author paper by Kare Berg in 1963 (Berg, 1963) and the subsequent association to cardiovascular disease (Berg et al. 1974), it has been anticipated that a specific therapy could be developed to lower Lp(a) levels and reduce cardiovascular risk. However, despite several nonspecific approaches, until now, it has been very difficult to potently and specifically lower plasma Lp(a) levels. The main impediments have been the fact that Lp(a) has no enzyme activity or receptor function, and therefore the only viable approach is to reduce synthesis and/or increase clearance of the actual particle. Clearance pathways are ill-defined and are mediated by multiple pathways and receptors whose individual quantitative contributions are not known. Additionally, they do not materially affect plasma levels, which are primarily influenced by the genetically driven synthetic capacity of the hepatocyte. The emergence of a novel therapeutic modality, interfering with messenger ribonucleic acid translation to make protein, has allowed potent and specific Lp(a) lowering therapy to become a reality (Crooke et al. 2018). This work will review the preclinical and clinical development of antisense oligonucleotides (ASOs) in the quest to develop a specific method to lower plasma Lp(a) and to test the "Lp(a) hypothesis," namely, that potently lowering Lp(a) will reduce risk of cardiovascular disease and aortic stenosis.

S. Tsimikas (✉)
Division of Cardiology, Sulpizio Cardiovascular Center, University of California San Diego, La Jolla, CA, USA
e-mail: stsimikas@health.ucsd.edu

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_22

Preclinical Proof of Concept in Lowering Plasma Lp(a) Levels

One of the limitations in developing modalities to lower Lp(a) in plasma has been the narrow species distribution of the *LPA* gene, limited to hedgehogs, Old World monkeys, apes, and humans (McLean et al. 1987; Tomlinson et al. 1989). Thus, appropriate animal models that can encompass the entire Lp(a) pathophysiology, including regulatory elements, have generally been lacking (Yeang et al. 2016).

One of the earliest reported attempts to interfere with apolipoprotein(a) biosynthesis was by Frank et al. (2001). They doubly transfected mice with an adenovirus-mediated N-terminal truncated apolipoprotein(a) construct comprising the 5′-untranslated region, the signal sequence, and the first three kringles of native apolipoprotein(a) along with a concomitant antisense molecule directed to apolipoprotein(a) mRNA. Evidence of transient but efficient reductions of apolipoprotein(a) synthesis was shown. However, this approach could not be easily translated to human applications due to the limitations of the animal models and the rapid degradation and presumed inefficiency of the antisense constructs used.

Several approaches have been shown to nonselectively and modestly lower Lp(a), including lipid apheresis, niacin, CETP inhibitors, and PCSK9 inhibitors (Tsimikas et al. 2021). The first proof-of-concept description of effective and long-lasting in vivo lowering of Lp(a) was reported by Merki et al. (2008) in 2008 in a collaborative effort of the Ionis Pharmaceuticals (then named Isis Pharmaceuticals) led by Mark Graham and Rosanne Crooke and our laboratory at UCSD (University of California, San Diego). In this study, Lp(a) lowering was achieved by the antisense oligonucleotide mipomersen, directed to human apoB-100, which significantly reduced human apoB-100 levels in Lp(a) transgenic mice expressing both human apoB-100 and apolipoprotein(a) needed to generate authentic Lp(a) particles. Over the 11-week treatment period, compared with baseline, mipomersen reduced Lp(a) levels by up to 75% ($p < 0.0001$) in a time-dependent fashion (Fig. 22.1a). This was primarily due to limiting the availability of apoB-100 to bind to apolipoprotein(a), as *LPA* mRNA expression and plasma apolipoprotein(a) levels were not affected by mipomersen. Furthermore, mipomersen significantly reduced plasma levels of oxidized phospholipids on apoB (OxPL-apoB) and apolipoprotein(a) [OxPL-apo(a)] particles (Fig. 22.1b). This study provided proof of concept that reducing the availability of apoB-100 is a limiting factor in Lp(a) particle assembly in this Lp(a) transgenic mouse model. These preclinical findings were later confirmed in clinical studies of mipomersen in a variety of settings, including in patients with homozygous and heterozygous familial and multifactorial hypercholesterolemia, showing approximately 25% reduction in Lp(a) that was independent of LDL-C lowering (Santos et al. 2015). The study also taught us that targeting apoB is not an ideal mechanism to lower Lp(a), as it does not affect the pathognomonic protein of Lp(a), apolipoprotein(a). Furthermore, the pathophysiological effect of free apolipoprotein(a), and whether it is more or less atherothrombotic and proinflammatory than Lp(a), is not known.

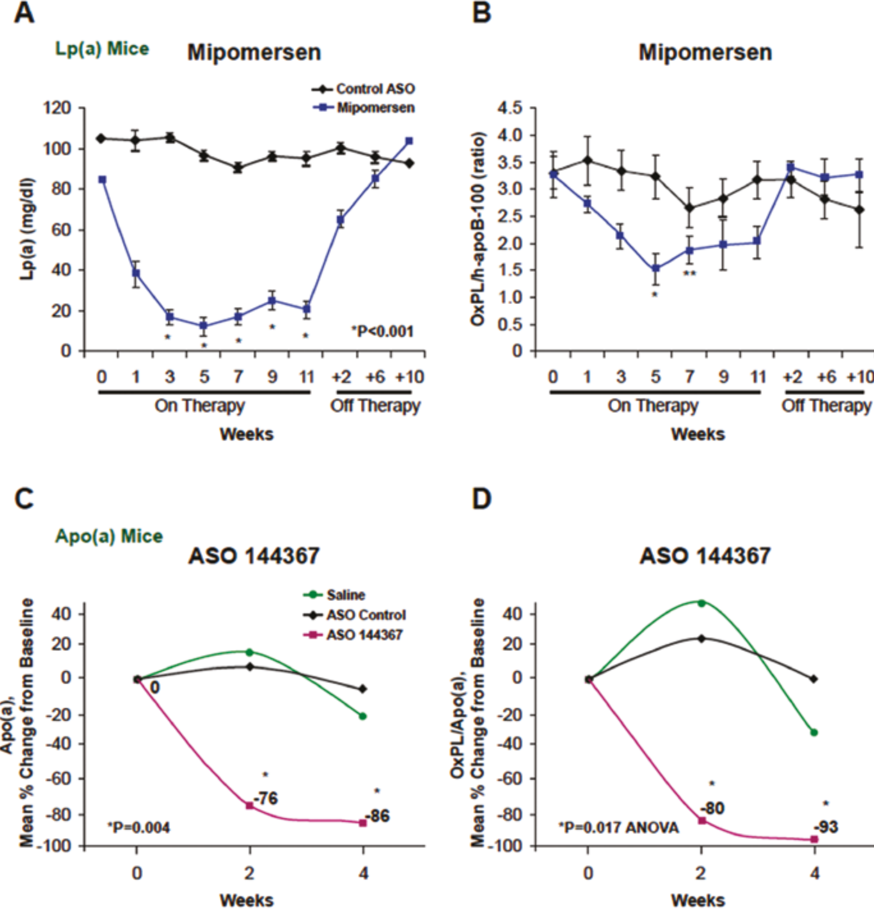


Fig. 22.1 Temporal changes in Lp(a) levels (A) and in OxPL-apoB levels in Lp(a)-transgenic mice treated with control ASO or mipomersen directed to human apoB. Temporal changes in apo(a) levels (A) and in OxPL-apo(a) levels in apo(a)-transgenic mice treated with saline, control ASO or ASO 144367. *P<001, **P<0.01 vs baseline values. Merki et al. 2008, 2011)

Due to the lack of apolipoprotein(a) reduction with mipomersen, a new approach was reported in 2011 using a specific antisense oligonucleotide, ISIS 144367, to target *LPA* mRNA (Merki et al. 2011). Three transgenic mouse models were utilized: 8K-apo(a) mice expressing eight kringle IV (KIV) repeats (KIV_1, with a single copy of KIV_2, a fusion of $KV_{3–5}$ and $KIV_{6–10}$), 8K-Lp(a) mice expressing both the 8K apo(a) and human apolipoprotein B-100, and 12K-apo(a) mice expressing a 12K apo(a) with three KIV_2 repeats. The mice were treated intraperitoneally with saline, a control ASO, or ASO 144367 directed to KIV_2 for 4–6 weeks. ASO 144367 significantly reduced Lp(a) by 24.8% in 8K-Lp(a) mice and reduced apolipoprotein(a) levels by 19.2% in 8K Lp(a) mice, 30.0% in 8K-apo(a) mice, and 86% in 12K-apo(a) mice (Fig. 22.1c). ASO 144367 also significantly reduced OxPL-apo(a) by 92.5%

in 8K-apo(a) mice (Fig. 22.1d). These studies provided proof that targeting liver expression of apolipoprotein(a) with ASOs directed to KIV_2 repeats may provide an effective approach to lower elevated Lp(a) levels in humans.

Evolution of Various Generations of Antisense Technology

Natural DNA and RNA are not suitable as effective drugs due to insufficient stability mediated by their rapid plasma degradation by nucleases and limited tissue distribution in animals. Antisense oligonucleotides (ASOs) are single-stranded modified DNA molecules comprised of 15–20 nucleic acids that display a complementary sequence to a target messenger ribonucleic acid (mRNA). To overcome the limitations of naked DNA/RNA as pharmaceutical agents, ASOs contain certain modifications of the phosphate backbone and the 2′ ribose position. ASOs have a specific sequence to the target of interest that is not repeated throughout genome, reducing the potential for off-target binding.

Using medicinal chemistry approaches, first-generation ASOs led to the substitution of phosphodiester (PO) bonds with phosphorothioate (PS) at one of the two available PO bonds at each phosphate group in the backbone to improve stability of the DNA:RNA complex and improve distribution to tissues. The PS bonds provide stability and protection against nucleases. Second-generation ASOs are called MOE-gapmers, in that the middle ten nucleic acids are unmodified DNA, which is required for RNAse H1-mediated cleavage, whereas the five nucleic acids on each wing are modified at the 2′ position by MOE. The 2′-MOE moiety increases stability in biological systems, increases potency due to improved binding affinity to its target mRNA, and improves the safety profile by decreasing proinflammatory effects and class toxicities. The nucleic acid bases are in their native chemical configuration and generally not modified in ASOs. Examples of 2′-MOEs are inotersen (Benson et al. 2018) that is approved clinically for hereditary amyloid polyneuropathy and volanesorsen (Witztum et al. 2019) that is approved clinically for familial chylomicronemia syndrome in the European Union.

A further advance was made in generation 2+ molecules by improving the screening process for proinflammatory and other undesirable side effects, as well as by removing some of the PS groups and replacing them with their native PO groups in the backbone. This class of drugs are represented by IONIS-APO(a)L_{Rx}, olezarsen (Tardif et al. 2022), targeting ApoCIII to treat hypertriglyceridemia and AGT-L_{Rx} (Morgan et al. 2021) targeting angiotensinogen for the proposed treatment of resistant hypertension and heart failure.

Additional improvements in antisense oligonucleotides include generation 2.5 molecules, where the 2′-MOE has been replaced by a constrained ethyl moiety, as exemplified by ION409/AZD8233 targeting PCSK9 (Gennemark et al. 2021). The changes in chemistry, from first generation to second generation and to generation 2.5, each improved potency by approximately tenfold and cumulatively by approximately 1000-fold, along with additional improvements in safety and tolerability (Crooke et al. 2018).

Finally, each of these modifications can be coupled with a triantennary *N*-acetylgalactosamine (GalNAc3) complex. GalNAc3 is a modified galactose moiety that is generated on proteins and aging cells and is a ligand for the asialoglycoprotein surface receptor (ASGPR) on hepatocytes. The clearance of GalNAc3 is an evolutionarily conserved mechanism for their removal from plasma by hepatocytes. This is a very high-capacity system in that each hepatocyte has up to one million asialoglycoprotein surface receptors (ASGPR) that allow rapid and specific uptake. This additional modification allows a further tenfold increase in potency.

Development of a Human Candidate to Lower Lp(a) Plasma Levels

There are two unique challenges in developing RNA-targeted therapeutics specifically for *LPA* mRNA. The first is that the *LPA* gene is very large (~10–15 kb depending on the number of KIV_2 repeats) and has 1–>40 KIV_2 repeats at the DNA/RNA level. The second is that the homology to plasminogen is 75–94% in the coding region. Both of these properties limit available sites for targeting ASOs without adversely affecting the coagulation system.

The screening process for identifying a human candidate is shown in Fig. 22.2. An in silico analysis is first performed for targeting sites, and ASOs are then designed with optimal predicted selectivity and tolerability. Next, multiple rounds of in vitro activity assessments are performed. For LPA screening, this involved both primary human hepatocytes and mouse hepatocytes obtained from transgenic mice expressing human *LPA*. Initial activity assessments involve evaluation of

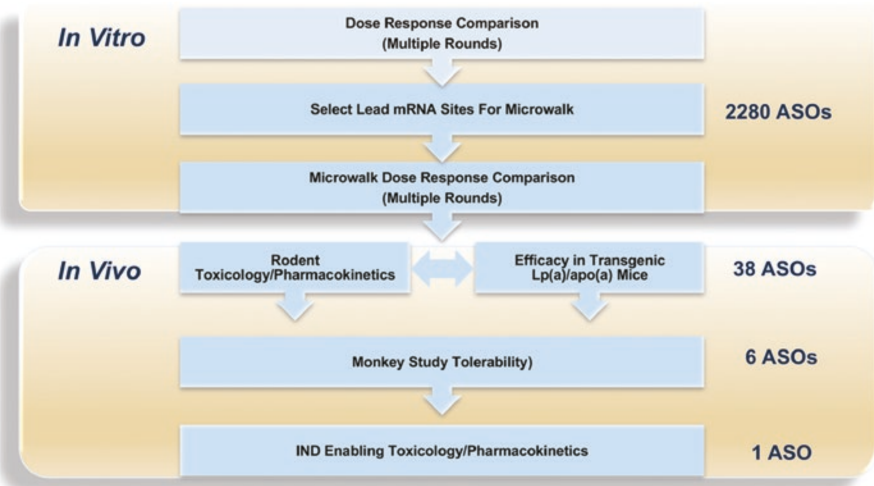


Fig. 22.2 Screening process to identify human antisense oligonucleotides to lower plasma Lp(a)

ASOs that span broad regions of the gene of interest. Top leads are then evaluated in dose-response comparisons with leads demonstrating the greatest potency selected for in vivo evaluation. Additionally, lead targeting sites are interrogated with "microwalks" to identify the most potent ASOs with an optimal safety and tolerability profile. In the case of identifying pelacarsen, this process required the synthesis of over 2800 unique ASOs prior to the identification of the clinical candidate. The most promising candidates, in this case 38 ASOs, were further interrogated for efficacy in Lp(a) or apo(a) transgenic mice and in rodent toxicology and pharmacokinetic studies. This process narrowed the choice to six potential candidates that were then evaluated in cynomolgus monkey tolerability studies, and the best candidate was identified to enter IND (Investigational New Drug) enabling toxicology and pharmacokinetic studies.

ISIS-APO(a)$_{Rx}$ (later named IONIS-APO(a)$_{Rx}$) is a second-generation 2′-*O*-(2-methoxyethyl) (2′-MOE)-modified ASO drug with the sequence 5′-TGCTCCGTTGGTGCTTGTTC-3′ (Fig. 22.3) (Graham et al. 2016). ISIS-APO(a)$_{Rx}$/IONIS-APO(a)$_{Rx}$ contains five 2′-MOE-modified ribonucleosides at the 5′ and 3′ ends and ten 2-*O*-deoxyribonucleosides within the central portion of the molecule. A modified version of IONIS-APO(a)$_{Rx}$ containing GalNAc3 was generated, initially named IONIS-APO(a)-L$_{Rx}$, and then AKCEA-APO(a)L$_{Rx}$ and ultimately received the generic name pelacarsen. Pelacarsen contains the same 20-nucleotide sequence as IONIS-APO(a)$_{Rx}$ and five 2′-MOE-modified ribonucleosides at the 5′

ISIS-APO(a)$_{Rx}$ Binding Site	Position on NM_005577.2 apo(a) mRNA transcript	Binding Site on First Exon	Binding Site on Second Exon
kringle IV2 repeat 2 Exon 4-5	505-524 bp	CTTGTTC	TGCTCAGTCGGTG
kringle IV2 repeat 3 Exon 6-7	847-866 bp	CTTGTTC	TGCTCAGTCGGTG
kringle IV2 repeat 4 Exon 8-9	1189-1208 bp	CTTGTTC	TGCTCAGTCGGTG
kringle IV2 repeat 5 Exon 10-11	1531-1550 bp	CTTGTTC	TGCTCAGTCGGTG
kringle IV2 repeat 6 Exon 12-13	1873-1892 bp	CTTGTTC	TGCTCAGTCGGTG
kringle IV2 repeat 7 Exon 14-15	2215-2234 bp	CTTGTTC	TGCTCAGTCGGTG
kringle IV2 repeat 8 Exon 16-17	2557-2576 bp	CTTGTTC	TGCTCAGTCGGTG
kringle IV2 repeat 9 Exon 18-19	2899-2918 bp	CTTGTTC	TGCTCAGTTGGTG
kringle IV2 repeat 11 Exon 22-23	3583-3602 bp	CTTCTTC	TGCTCCGTTGGTG
kringle IV2 repeat 12 Exon 24-25	3901-3920 bp	CTTGTIC	TGCTCCGTTGGTG
kringle IV2 repeat 14 Exon 28-29	4584-4604 bp	CTTGTIC	TTCTCAGGTGGTG
kringle IV2 repeat 15 Exon 30-31	4927-4946 bp	CTGCTTC	TGCTCAGTGGTG

Fig. 22.3 ISIS-APO(a)$_{Rx}$ complementary binding sites within the human *LPA* transcript (GenBank accession NM_005577.2) at position 3901–3920 bp. ISIS-APO(a)$_{Rx}$ was designed to perfectly match only the exon 24–25 splice sites (indicated with bold type) but may also bind at 11 other apolipoprotein(a) exon splice sites containing 1–4 mismatched nucleotides (indicated by underlined letters) (Graham et al. 2016)

and 3′ ends while retaining ten 2-odeoxyribonucleosides within the central portion of the molecule. However, 6 of the 19 PS linkages were replaced with PO linkages at positions 2, 3, 4, 5, 16, and 17. The GalNAc3 complex was covalently attached with a proprietary linker to the 5′ end (Viney et al. 2016) (Fig. 22.4).

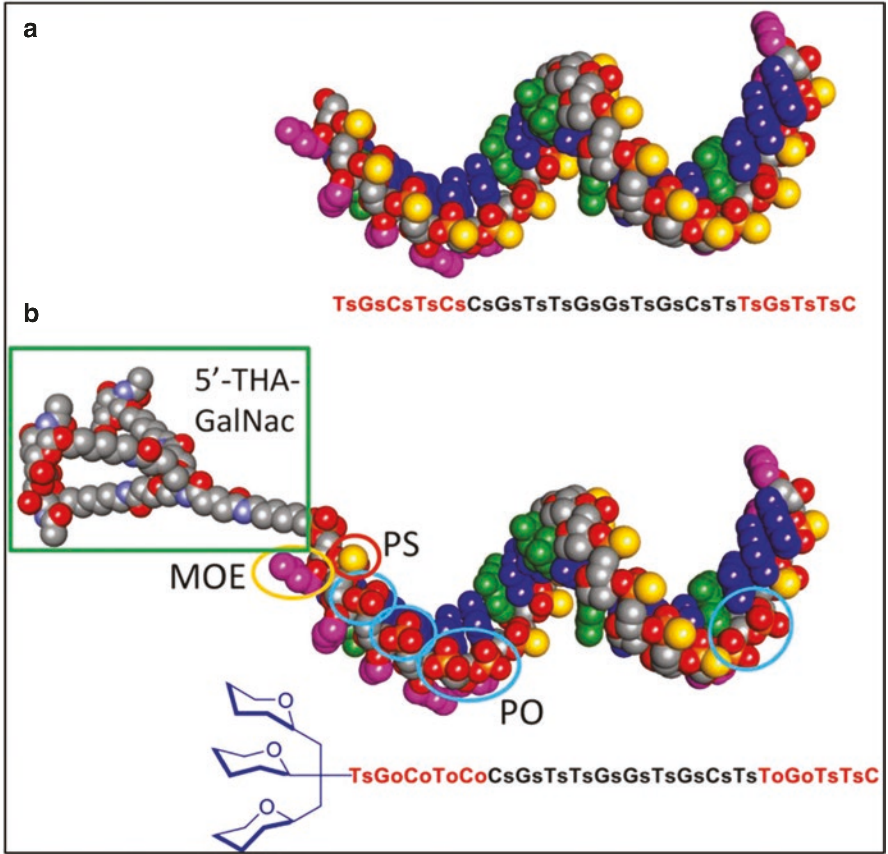


Fig. 22.4 Structure and sequence of ISIS-APO(a)*Rx*/IONIS-APO(a)$_{Rx}$ (**a**) and IONIS-APO(a)-L$_{Rx}$/AKCEA-APO(a)L$_{RX}$/TQJ230/pelacarsen (**b**). The figure depicts space-filling models with the nucleic acid sequence in capital letters below. IONIS-APO(a)$_{Rx}$ contains 20 nucleic acids with ten 2-*O*-deoxyribonucleosides within the central portion of the molecule and incorporates five 2′-methoxyethyl (MOE)-modified ribonucleosides at the 5′ and 3′ ends. IONIS-APO(a)-L$_{Rx}$ (pelacarsen) contains the same nucleic acid sequence but has only 6 of the 19 sulfur groups in the backbone and additionally contains the triantennary *N*-acetylgalactosamine (GalNAc3) complex. The phosphodiester (PO) linkages are indicated by an oxygen (O) for native linkage and by a sulfur (S) for phosphorothioate (PS) substitution. The GalNAc3 complex is attached to the 5′ end via a proprietary THA linker for rapid and specific uptake within hepatocytes via the asialoglycoprotein receptor (Viney et al. 2016)

Mechanisms of Antisense Oligonucleotide Pharmaceutical Activity

The mechanism of efficacy of ASOs utilizes the ubiquitous intracellular ribonuclease RNase H1 that recognizes the RNA:DNA duplex formed when ASOs bind to the complementary mRNA sequence. RNAse H1 binds to this duplex, irrespective of the

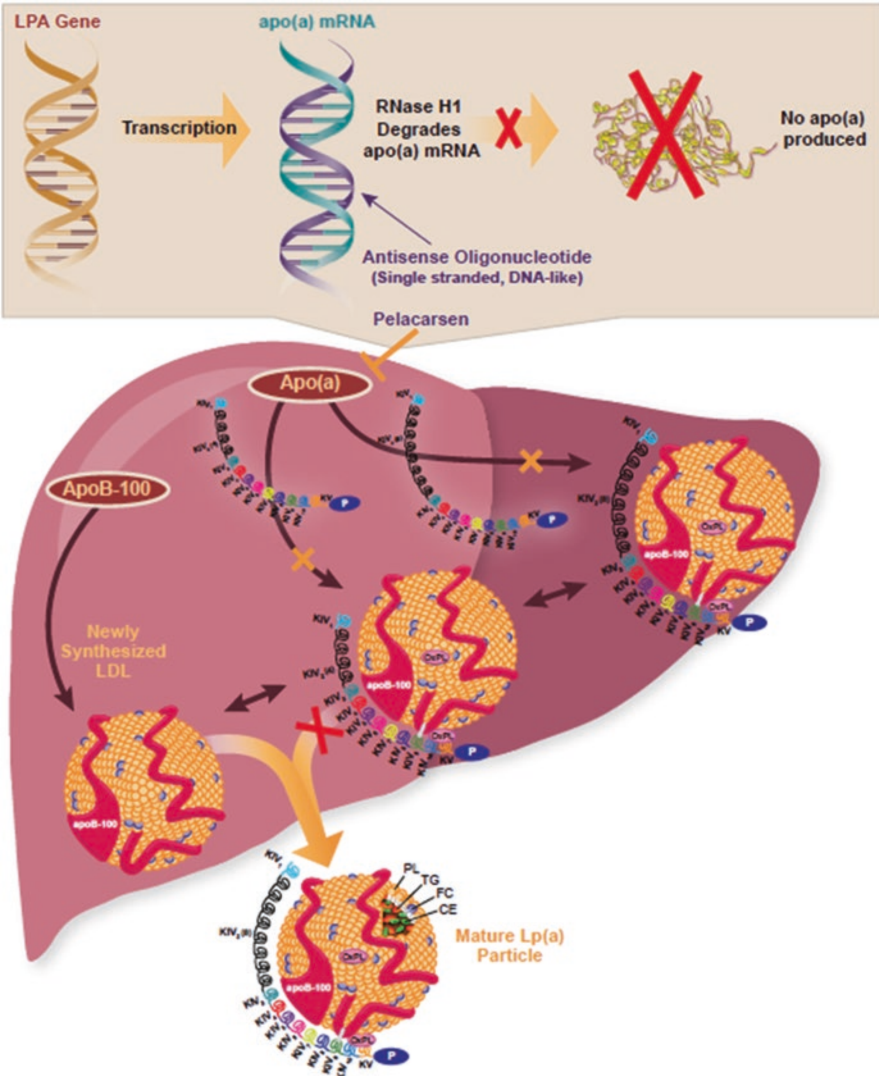


Fig. 22.5 Mechanism by which *LPA*-directed antisense oligonucleotides suppress apolipoprotein(a) protein synthesis. Following formation of the *LPA* mRNA:pelacarsen duplex, the ubiquitous intracellular ribonuclease RNase H1 recognizes the duplex and cleaves the target *LPA* mRNA sense strand, thereby preventing translation of apolipoprotein(a) protein. The hepatocytes continue to generate apoB-100 particles, but the relative absence of apolipoprotein(a) does not allow the assembly of Lp(a) particles (Viney et al. 2016)

specific sequence of the ASO or the target, and cleaves the target mRNA, thereby disrupting protein translation (Fig. 22.5). The ASO is relatively resistant to RNAse H1-mediated cleavage and becomes available to bind to additional mRNA *LPA* molecules. This is part of reason the intra-hepatocyte half-life is relatively long (2–4 weeks).

Completed Clinical Trials with ISIS-APO(a)$_{Rx}$/ IONIS-APO(a)$_{Rx}$

A total of four clinical phase 1 or phase 2 trials have been performed with ISIS-APO(a)$_{Rx}$/IONIS-APO(a)$_{Rx}$/and pelacarsen (Table 22.1). For historical purposes and to be consistent with the literature, the names of the drugs will be given according to those used when the trials were published.

Table 22.1 Competed clinical trials with antisense oligonucleotides

Study	Year published	Drug	Number of subjects	Dose/dose regimen	Mean baseline Lp(a), (nmol/L)	Mean Lp(a) reduction	Absolute Lp(a) reduction, (nmol/L)
Tsimikas et al. (2015)	2015	ISIS-APO(a)$_{Rx}$	16	Single doses of 50, 100, 200, and 400 mg	8–66	No significant change	N/A
		ISIS-APO(a)$_{Rx}$	31	100, 200, and 300 mg/week, six doses over 4 weeks	82–152	40–78%	34–95
Viney et al. (2016)	2016	IONIS-APO(a)$_{Rx}$	Cohort A—50	100–300 mg/week for 13 weeks	252–254	67%	183
		IONIS-APO(a)$_{Rx}$	Cohort B—11	100–300 mg/week for 13 weeks	445–488	72%	305
Viney et al. (2016)	2016	Pelacarsen	28	Single doses of 10, 20, 40, 80, and 120 mg	111–219	26–85%	59–107
		Pelacarsen	30	Multiple doses 10, 20, and 40 mg/week for 4 weeks	143–165	66–92%	86–141
Tsimikas et al. (2020)	2020	Pelacarsen	286	20, 40, or 60 mg every 4 weeks, 20 mg every 2 weeks, or 20 mg every week for 6–12 months	205–247	35–80%	96–188

Lp(a) molar concentration in nmol/L cannot be scientifically converted to mass units in mg/dL. However, a rough estimate is to divide nmol/L by 2.5 to approximate values in mg/dL, with the realization that significant error may occur depending on isoform size (Tsimikas et al. 2018)

The first clinical demonstration that Lp(a) levels can be potently reduced in patients was documented in a randomized, double-blind, placebo-controlled, phase 1 study of healthy adults with Lp(a) concentration of ≥25 nmol/L assigned to receive ISIS-APO(a)$_{Rx}$ or placebo. Multiple doses of ISIS-APO(a)$_{Rx}$ (100–300 mg) resulted in dose-dependent, mean percentage decreases in plasma Lp(a) concentration of 39.6% from baseline in the 100 mg group ($p = 0.005$), 59.0% in the 200 mg group ($p = 0.001$), and 77.8% in the 300 mg group ($p = 0.001$) (Fig. 22.6). Similar reductions were observed in OxPL-apoB and OxPL-apo(a) (Fig. 22.6). Mild injection site reactions were the most common adverse events. No serious or severe adverse events were recorded. Two of the 37 participants treated with ISIS-APO(a)$_{Rx}$ discontinued the study drug for tolerability reasons, which was an improved experience compared to previous earlier drugs in this class of chemicals.

ISIS-APO(a)$_{Rx}$ was renamed IONIS-APO(a)$_{Rx}$ in concert with the change in the company's name. The phase 2 study that followed was performed in participants with elevated Lp(a) concentrations (125–437 nmol/L in cohort A with 51 participants, ≥438 nmol/L in cohort B with 13 participants) who were randomly assigned to escalating-dose subcutaneous IONIS-APO(a)$_{Rx}$ (100 mg, 200 mg, and then 300 mg, once a week for 4 weeks each) or saline placebo, once a week, for 12 weeks. At day 85/99, participants assigned to IONIS-APO(a)$_{Rx}$ had mean Lp(a) reductions of 66.8% in cohort A and 71.6% in cohort B (both $p < 0.0001$ vs pooled placebo)

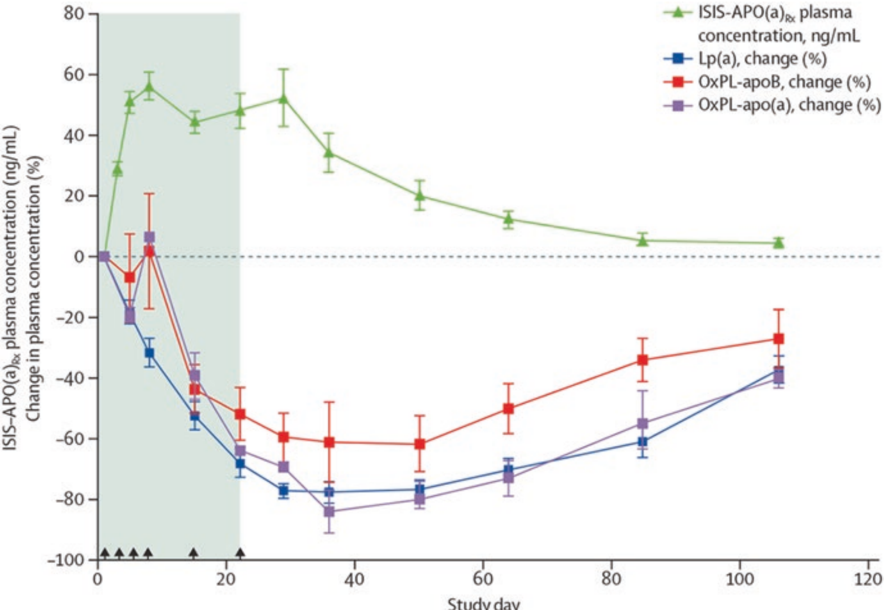


Fig. 22.6 Plasma trough concentrations of ISIS-APO(a)$_{Rx}$ and mean percent change in Lp(a), OxPL-apoB, and OxPL-apo(a) with time by treatment group in the multidose cohorts measured 7 days after the last dose in the 300 mg dose cohort in relation to change in concentration of plasma Lp(a), OxPL-apoB, and OxPL-apo(a). The shaded area represents the dosing window, and arrows indicate dosing at days 1, 3, 5, 8, 15, and 22 (Tsimikas et al. 2015)

(Fig. 22.7). Mean concentrations were also reduced in OxPL-apoB (35.2% for cohort A and 42.5% for cohort B), OxPL-apo(a) (26.6% for cohort A and 36.7% for cohort B), LDL-C (13.0% for cohort A and 23.9% for cohort B), and apoB-100 (11.3% for cohort A and 18.5% for cohort B) (Fig. 22.7).

Baseline hsCRP concentrations were 2.39 mg/L for the placebo group, 1.78 mg/L for cohort A, and 3.46 mg/L for cohort B. At day 85/99, mean absolute change in hsCRP was −0.64 mg/L (SD 4.38, $p = 0.44$ vs baseline) for the pooled placebo group, −0.23 mg/L for cohort A (SD 1.54, $p = 0.92$ vs baseline and $p = 0.63$ vs change for placebo), and − 1.5 mg/L for cohort B (SD 3.27, $p = 0.37$ vs baseline and $p = 0.20$ vs change in placebo). Overall, IONIS-APO(a)$_{Rx}$ was generally well

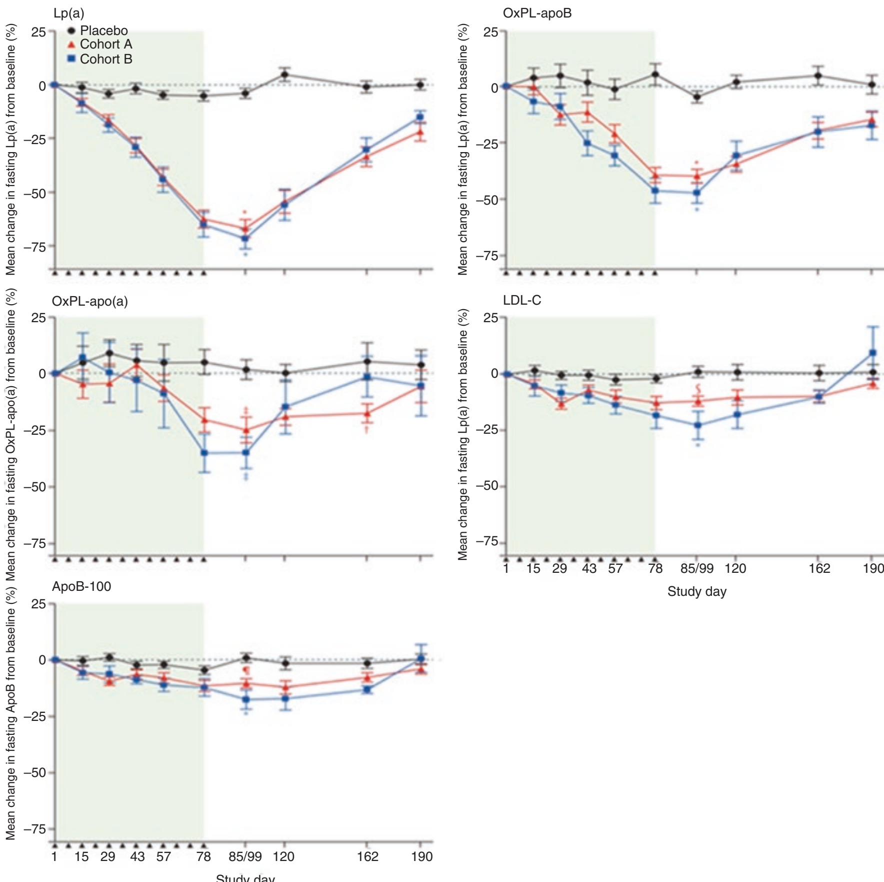


Fig. 22.7 Mean percent changes in plasma concentrations of Lp(a), OxPL-apoB, OxPL-apo(a), LDL-C, and apoB in the IONIS-APO(a)$_{Rx}$ trial. Error bars are SEM (standard error of the mean). The shaded area represents the dosing window and arrows indicate dosing every week. *p* values show differences between treatment and pooled placebo at day 85/99. $^{*}p \leq 0.0001$, $^{\dagger}p = 0.0002$, $^{\ddagger}p = 0.0005$, $^{\S}p = 0.0007$, $^{\P}p = 0.0003$ (Viney et al. 2016)

tolerated with 10% of injections in cohort A and 19% of injections in cohort B associated with injection site reactions (overall 12%). Approximately 10% of patients had individual components that could be consistent with influenza-like symptoms.

Completed Clinical Trials with IONIS-APO(a)L_{Rx}/AKCEA-APO(a)L_{Rx},/TQJ230/Pelacarsen

The rapid development of hepatocyte-targeting technology, with the promise of lower dosing and improved safety and tolerability, led to the decision to switch clinical development to IONIS-AP0(a)L_{Rx}/AKCEA-APO(a)L_{Rx},/TQJ230/pelacarsen, all of which are the same molecule (the generic name pelacarsen will be used subsequently). A new phase 1/phase 2a study was initiated in 58 healthy participants with doses of 10, 20, 40, 80, and 120 mg in the single-ascending-dose phase and 30 participants of 10, 20, and 40 mg weekly in the multiple-ascending-dose phase. Significant dose-dependent reductions in mean Lp(a) concentrations were noted in all single-dose pelacarsen groups at day 30 with mean reductions at day 30 of 26.2% in the 10 mg group, 33.2% in the 20 mg group, 43.5% in the 40 mg group, 78.6% in the 80 mg group, and 85.3% in the 120 mg group versus a 2.8% mean increase in the placebo group (Fig. 22.8a). In the multidose groups, pelacarsen resulted in mean reductions in Lp(a) of 66% in the 10 mg group, 80% in the 20 mg group, and 92% in the 40 mg group ($p = 0.0007$ for all vs placebo) at day 36 (Fig. 22.8b). Pelacarsen was associated with no injection site reactions. Compared to IONIS-APO(a)$_{Rx}$, pelacarsen was documented to be approximately 30-fold more potent (Fig. 22.8c).

This trial provided the proof of concept that GalNAc-modified ASOs could be targeted to the hepatocyte in a safe and tolerable manner and that potency could be substantially increased. A phase 2 dose-ranging trial with dosing regimens ranging from weekly to monthly was then performed in 286 patients with established cardiovascular disease and screening Lp(a) levels of ≥60 mg/dL (≥150 nmol/L) (Tsimikas et al. 2020). The median baseline Lp(a) levels in the six groups ranged from 204.5 to 246.6 nmol/L. Administration of pelacarsen resulted in dose-dependent decreases in Lp(a) levels, with mean percent decreases of 35% at a dose of 20 mg every 4 weeks, 56% at 40 mg every 4 weeks, 58% at 20 mg every 2 weeks, 72% at 60 mg every 4 weeks, and 80% at 20 mg every week, as compared with 6% with placebo (p values for the comparison with placebo ranged from 0.003 to <0.001, Fig. 22.9a). There were no significant differences between any pelacarsen dose and placebo with respect to platelet counts, liver and renal measures, or influenza-like symptoms. The most common adverse events were injection site reactions.

The temporal changes in Lp(a) levels reveal significant declines as early as 4 weeks post first injection, reaching a steady state at approximately 16 weeks

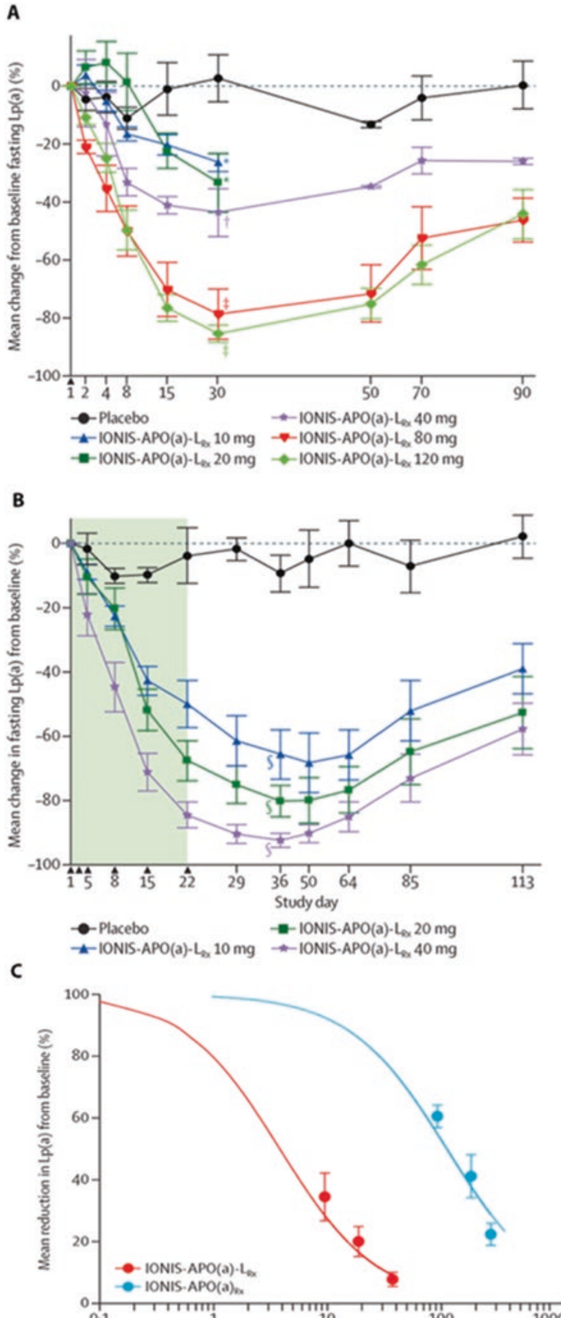
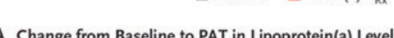
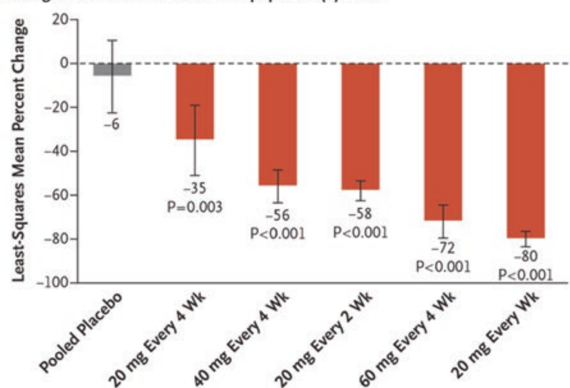
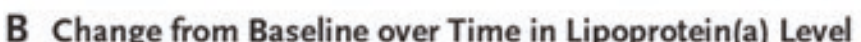
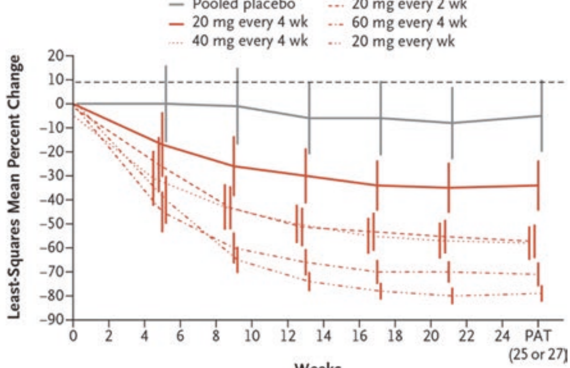


Fig. 22.8 Mean percentage change in Lp(a) concentration in the IONIS-APO(a)-L_{Rx} trial and comparison of dose-response curves of IONIS-APO(a)$_{Rx}$ and IONIS-APO(a)-L_{Rx}. (**a**) Single-ascending-dose and (**b**) multiple-ascending-dose phases. The shaded area represents the dosing window and arrows indicate dosing days. *p* values are only shown at day 30 for the single-ascending-dose phase and day 36 for multiple-ascending-dose phase as determined by the exact Wilcoxon rank-sum test comparing IONIS-APO(a)-LRx versus placebo. $^{*}p = 0.0333$, $^{\dagger}p = 0.0167$, $^{\ddagger}p = 0.0012$, $^{\S}p = 0.0007$. (**c**) Comparison of dose-response curves of IONIS-APO(a)$_{Rx}$ and IONIS-APO(a)-L_{Rx} after 4 weeks of subcutaneous administration. Error bars are SEM. The upper left side of the curve was extrapolated based on the curve fit of the data due to the fact that lower doses were not tested (Viney et al. 2016)









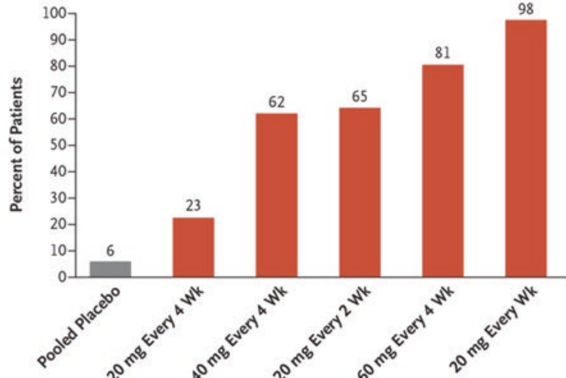


Fig. 22.9 Effect of pelacarsen (AKCEA-APO(a)-L$_{Rx}$) on Lp(a) levels. Panel (**a**) shows the least squares mean percent changes from baseline to the primary analysis time point (PAT) (i.e., 6 months of exposure [at week 25 in the groups who received monthly doses and at week 27 in the groups who received more frequent doses]). I bars denote 95% confidence intervals. Panel (**b**) shows the least squares mean percent changes from baseline in Lp(a) over time. Error bars denote 95% confidence intervals. Panel (**c**) shows the percent of patients with Lp(a) levels of <50 mg/dL (<125 nmol/L) in each group at the PAT (Santos et al. 2015)

(Fig. 22.9b). The percent of patients with an Lp(a) level of ≤50 mg/dL (125 nmol/L) or lower at 6 months of exposure was 23% in the group who received 20 mg of pelacarsen every 4 weeks, 63% in the group who received 40 mg every 4 weeks, 65% in the group who received 20 mg every 2 weeks, 81% in the group who received 60 mg every 4 weeks, and 98% in the group who received 20 mg every week (Fig. 22.9c).

The mean percent reductions in OxPL-apoB were 37% at a dose of 20 mg every 4 weeks, 57% at 40 mg every 4 weeks, 64% at 20 mg every 2 weeks, 79% at 60 mg every 4 weeks, and 88% at 20 mg every week, as compared with a 14% increase in the placebo group. The corresponding mean percent reductions in OxPL-apo(a) were 28%, 49%, 45%, 63%, and 70%, respectively, compared with a 20% decrease in the placebo group. Corresponding absolute reductions in LDL-C were −5.6, −13.5, −13.2, −8.2, and −16.4 mg/dL, respectively, compared to −1.2 mg/dL in placebo. Corresponding absolute reductions in apoB were −2.2, −8.3, −6.3, −3.9, and −10.9 mg/dL, respectively, compared to 0.6 mg/dL increase in placebo. Corresponding absolute changes (nonsignificant) in hsCRP were −0.9, −0.7, −0.3, −0.5, and −0.1 mg/L, respectively, compared to −0.8 mg/L in placebo.

Individual responses revealed that all patients in the pelacarsen 20 mg weekly dose had declines in Lp(a) (−42.6 to −99.5%), OxPL-apoB (−37.0 to 99.7%), and OxPL-apo(a) (−12.6 to −99.5%), compared to +16.1 to −40.6%, −28.7 to +150.0%, and −66.6 to +18.1 for the three variables in the placebo groups, respectively.

There were no significant differences between any pelacarsen dose and placebo with respect to platelet counts, liver and renal measures, or influenza-like symptoms. The most common adverse events were injection site reactions.

Lp(a) Horizon Cardiovascular Outcomes Trial and Testing of the "Lp(a) Hypothesis"

Based on the totality of epidemiologic, genetic, and clinical evidence, as well as the proof of concept in potently lowering Lp(a) these four trials provided, further development of pelacarsen was undertaken. The Lp(a) HORIZON trial (NCT04023552, Assessing the Impact of Lipoprotein(a) Lowering With Pelacarsen on Major Cardiovascular Events in Patients With CVD [Lp(a) HORIZON] is a pivotal phase 3 study designed to support an indication for the reduction of cardiovascular risk in patients with established CVD and elevated Lp(a). It is a global, international multicenter, randomized, double-blind, placebo-controlled study in >8000 patients with elevated Lp(a) levels (≥70 mg/dL, ≥175 nmol/L) and history of CVD (myocardial infarction, ischemic stroke, peripheral artery disease). Key inclusion criteria include 1-Lp(a) ≥70 mg/dL, 2-myocardial infarction ≥3 months from screening and randomization to ≤10 years prior to the screening visit, 3-ischemic stroke ≥3 months from screening and randomization to ≤10 years prior to the screening visit, and clinically significant symptomatic peripheral artery disease.

Subjects are required to have risk factors, particularly LDL-C, optimized according to local guidelines. They are then randomized to pelacarsen 80 mg

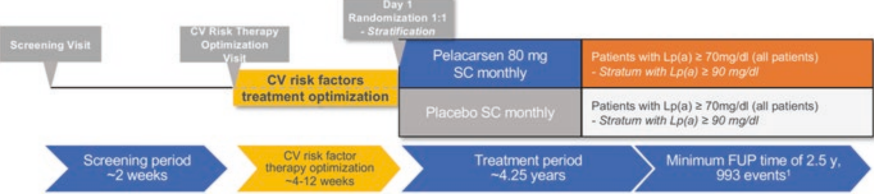


Fig. 22.10 Design of the Lp(a) HORIZON trial

subcutaneously monthly versus matching placebo. The primary outcome measure is the time to first occurrence of expanded major adverse cardiovascular events (MACE), consisting of cardiovascular death, nonfatal MI, nonfatal stroke, and urgent coronary revascularization requiring hospitalization in the overall study population with established CVD and Lp(a) ≥70 mg/dL. A co-primary outcome measure is the time to first occurrence of expanded MACE, in the overall study population with established CVD in the overall study population and Lp(a) ≥90 mg/dL (Fig. 22.10). Secondary outcome measures include (1) the time to the first occurrence of MACE (CV death, nonfatal MI, and nonfatal stroke), (2) the time to first occurrence of the composite endpoint of coronary heart disease (coronary heart disease death, nonfatal MI, urgent coronary revascularization requiring hospitalization), and (3) the number of participants with confirmed all-cause death.

In conclusion, targeting *LPA* mRNA with antisense oligonucleotides is a fundamentally new approach to potently reducing circulating Lp(a) levels. The Lp(a) HORIZON trial is testing the "Lp(a) hypothesis," namely, that lowering plasma Lp(a) levels will lead to a reduced rate of recurrent cardiovascular events. It is expected to have primary results in 2025.

Acknowledgements We thank Rosanne Crooke, Mark Graham, Richard Geary, Nick Viney, and other members of the Ionis Pharmaceuticals and Joseph L. Witztum for their contributions in discovering and developing pelacarsen and Trace Reigle of the Ionis Pharmaceuticals for the preparation of some of the artwork.

Disclosures ST is a coinventor and receives royalties from patents on oxidation-specific antibodies and of biomarkers related to oxidized lipoproteins held by UCSD. ST is a co-founder and has equity interest in Oxitope, Inc., and its affiliates ("Oxitope") as well as in Kleanthi Diagnostics, LLC ("Kleanthi"). The terms of this arrangement have been reviewed and approved by the University of California, San Diego, in accordance with its conflict-of-interest policies. ST has a dual appointment at the UCSD and Ionis Pharmaceuticals.

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Chapter 23
Lipoprotein Apheresis for Reduction of Lipoprotein(a)

Ulrich Julius and Sergey Tselmin

Abbreviations

A	Atorvastatin
AIC	Arteria iliaca communis
Aorta-mes bypass	Mesenteric artery bypass
A. mes sup	Arteria mesenterica superior
ApoE	Apolipoprotein E-polymorphism
BMS	Bare metal stent
CAD	Coronary artery disease
CX	Left circumflex coronary artery
DEB	Drug-eluting balloon
DES	Drug-eluting stent
LAD	Left anterior descending artery
LCA	Left coronary artery
LMS	Left main stem
nk	Not known
MI	Myocardial infarction
PLA	Posterolateral artery
PTA	Percutaneous transluminal angioplasty
PTCA	Percutaneous transluminal coronary angioplasty
RCA	Right coronary artery
RDI	Ramus diagonalis, diagonal branch 1
R	Rosuvastatin

U. Julius (✉) · S. Tselmin
Department of Internal Medicine III, Lipidology and Center for Extracorporeal Treatment, University Hospital Carl Gustav Carus at the Technische Universität Dresden, Dresden, Germany
e-mail: ulrich.julius@ukdd.de

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_23

S	Simvastatin
TEA	Thromboendarteriectomy
Tr. coel	Truncus coeliacus

Introduction

An elevation of lipoprotein(a) [Lp(a)] is an internationally recognized risk factor for cardiovascular events (CVEs) such as myocardial infarction, stroke, or peripheral arterial disease (Nordestgaard et al. 2010; Tsimikas 2017; Reyes-Soffer et al. 2022; Kronenberg 2022). Statins usually do not have an impact on Lp(a) levels or even may increase them (Reyes-Soffer et al. 2022; Korneva et al. 2021). Niacin is no longer available; ezetimibe and bempedoic acid do not exert any effect on Lp(a). On the other hand, monoclonal antibodies directed against PCSK9 (alirocumab, evolocumab) decrease Lp(a) concentrations by up to 30% (Julius et al. 2019). In two prospectively conducted placebo-controlled intervention studies (Fourier, ODYSSEY OUTCOMES), it could be shown that the lower Lp(a) levels in the verum groups led to a reduction in CVEs, independent of the effect on LDL cholesterol (LDL-C) (Szarek et al. 2020; O'Donoghue et al. 2019). In studies with inclisiran, a similar decrease of Lp(a) was seen (up to 26%); outcome data are not yet available (Wright et al. 2021). But PCSK9 inhibitors are not officially accepted for the indication of an elevation of Lp(a), and they are not effective in this direction in all patients.

Thus, lipoprotein apheresis (LA) is at present the only therapeutic approach which has been shown to effectively reduce Lp(a) concentrations and CVEs. This article describes the current knowledge about the impact of LA on lipid concentrations and cardiovascular outcomes. One major advantage of LA in comparison with drug treatment is that the extracorporeal therapy is rather well tolerated. Besides heparin and/or citrate, isotonic saline solution, and in some cases calcium, no other foreign substances are applied in the daily routine of an apheresis center. In the 1960s, a plasma exchange was performed to treat patients with homozygous familial hypercholesterolemia (Thompson and Parhofer 2019)—albumin or fresh frozen plasma coming from donors substituted the plasma of the patients. Modern LA methods usually do not induce a loss of blood, and the plasma components which are removed do not have to be replaced.

History of Lipoprotein Apheresis (LA) Regarding the Indication of High Lipoprotein(a) [Lp(a)]

Between 1975 and 2004, several LA systems were developed to treat patients with familial hypercholesterolemia (Julius 2017; Thompson 2022). Lp(a) appeared as a target molecule only after 2000.

The largest number of patients treated with LA worldwide exists in Germany. In 2008, the Joint Federal Committee (Gemeinsamer Bundesausschuss), a paramount

decision-making body of the German Healthcare System, accepted an elevation of Lp(a) as an indication for LA (Bundesministerium für Gesundheit 2008). In the anteceding years, several patients were treated after courts had supported this indication in high-risk patients.

An Lp(a) level exceeding 60 mg/dL was set as the threshold value at which LA is indicated. Comparative measurements in large apheresis centers established 120 nmol/L as equivalent (Schettler et al. 2015).

Moreover, the following two additional conditions had to be fulfilled

1. The LDL-C concentration should be optimized (by drugs when tolerated; in some patients, this requirement is difficult to be met).
2. A progress of atherosclerosis needed to be documented either clinically (several CVEs) or by imaging techniques (in the clinical practice, the first myocardial infarction in a young patient with a positive family history for CVEs is accepted as an indication; some specialists regard a CVE already as progress per se).

In the United States, the Food and Drug Administration's approval specifically for Lp(a) lowering also requires an Lp(a) >60 mg/dL (about 150 nmol/L) (Reyes-Soffer et al. 2022).

The HEART UK Lipoprotein apheresis guidelines recommend that apheresis should be considered for those patients with progressive coronary disease and Lp(a) greater than ~150 nmol/L (>60 mg/dL) whose LDL-C remains 3.3 mmol/L or higher despite maximal lipid-lowering therapy (Cegla et al. 2019). The attitude toward LDL-C differs from that in Germany.

In Russia, LA is recommended for patients with homozygous or heterozygous familial hypercholesterolemia in combination with Lp(a) >60 mg/dL and early onset of atherosclerotic cardiovascular disease (Pokrovsky et al. 2020).

As far as the authors know, other countries where this indication is recognized are Italy (Stefanutti et al. 2013, 2020) and Poland (Mickiewicz et al. 2021).

It has to be emphasized that an LA treatment is only justified in patients within a secondary prevention strategy, meaning that CVEs had occurred or severe progressive atherosclerosis is documented. There is no indication to treat patients extracorporeally just to reduce elevated Lp(a) concentrations in a primary prevention concept.

In the last years, the number of patients who were treated with LA has continuously increased in Germany. The approval for the reimbursement of costs by the health insurance companies is based on an individual application to be renewed annually to regionally appointed committees of the Associations of Statutory Health Insurance Physicians. This major hurdle represents an important step of quality control for strict selection of patients. The rate of refusals to accept the application is rather high, especially among patients with the diagnosis "isolated elevation of Lp(a)." These numbers are also published for the federal states of Germany (in Table 23.1, only basic data are given). The number of physicians who got the permission to perform an LA therapy amounted to 1286 in the end of 2020.

Table 23.1 Numbers of patients treated with LA at the end of the given years in Germany

Year	2013	2014	2015	2016	2017	2018	2019	2020
hoFH	187	120	122	103	89	97	93	95
Severe HCH	1221	1472	1597	1700	1538	1477	1575	1663
Isolated Lp(a) elevation	753 (35 %)	954 (37 %)	1303 (43 %)	1468 (45 %)	1895 (54 %)	2163 (57 %)	2396 (59 %)	2448 (58 %)
Total	2161	2546	3022	3271	3522	3737	4064	4206

Data are officially published by the National Association of Statutory Health Insurance Physicians (Kassenärztliche Bundesvereinigung) (Kassenärztliche Bundesvereinigung 2014, 2015, 2016, 2017, 2018, 2019, 2020, 2021)
hoFH homozygous familial hypercholesterolemia, *HCH* hypercholesterolemia

The percentage of patients who were included into LA treatment with the indication of an isolated Lp(a) elevation was increasing from 35% in 2013 to 59% in 2019. At the Dresden Center for Extracorporeal Therapy, in the last years, the vast majority of new patients had elevations of Lp(a). Unfortunately, our 160 patients are not included into the data shown in Table 23.1 because working in a university hospital, we do not belong to the National Association of Statutory Health Insurance Physicians.

Efficiency of LA with Respect to Lowering of Lp(a) Concentrations

An LA session acutely reduces lipid concentrations which rise again in the following days. Thus a sawtooth picture is seen. In our experience, after 7 days, the Lp(a) level is again at its maximum.

In order to describe the efficiency of LA on Lp(a) levels, the following criteria are used

1. Acute reductions—comparing the Lp(a) level after LA sessions with those before the sessions.
2. Interval mean values—taking into account that Lp(a) increases following an LA session. This parameter defines the burden imposed by Lp(a) on arteries. We have published a formula to calculate interval mean values (Tselmin et al. 2017).
3. Comparison of the presession and post-session and interval mean values with the initial Lp(a) concentrations measured before the first LA session (reflecting the steady-state condition till the start of the extracorporeal therapy).

The following data have been measured at our center in 2019; the first LA session usually took place years ago (Julius et al. 2020). The vast majority of our patients are treated with LA weekly.

In our patients, the acute reductions amounted to 128 nmol/L (−77%) (see Table 23.2). When comparing with the initial Lp(a) level before the first LA session, the interval mean values were decreased by 134 nmol/L (−55%). Waldmann and

Table 23.2 Lp(a) before the first LA session, before and after an LA session, and interval mean values after years of extracorporeal therapy—percent reductions comparing with the initial levels are marked in red ($n = 97$; data have been recalculated on the basis of a publication in 2020) (Julius et al. 2020)

Timepoint of blood sampling	Before 1st LA	Before LA session after years	After LA session after years	Interval mean values after years
Median (nmol/l) / Percent reduction comparing with Lp(a) before 1st LA	242 / baseline	167 / - 31 %	39 / - 84 %	108 / - 55 %
IQR (nmol/l)	192 / 308	127 / 212	29 / 53	84 / 152
Min / Max (nmol/l)	70 / 820	50 / 391	10 / 122	27 / 288

IQR interquartile range

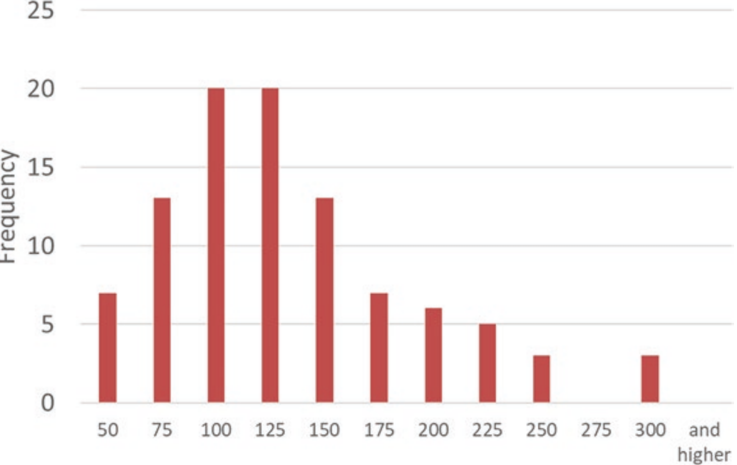


Fig. 23.1 Interval mean values of Lp(a) ($n = 97$; x-axis: upper bounds of intervals, nmol/L)

Parhofer reported a decrease of this parameter between 25 and 40% (Waldmann and Parhofer 2016). Evidently, the results obtained in Dresden are superior to these data. But it has to be taken into account that an additional Lp(a)-lowering effect may be due to the application of PCSK9 antibodies in some patients at our center.

The following histogram (Fig. 23.1) shows the interval mean values of Lp(a). Only in 20 (21%) patients the interval mean value was below 75 nmol/L.

Almost half of the patients showed interval mean values above 120 nmol/L.

The corresponding LDL-C interval mean value [calculated according to Kroon et al. (2000)] was 1.75 mmol/L (IQR 1.32/2.22 mmol/L), which is −15% lower than the initial level [2.07 mmol/L (IQR 1.77/3.04 mmol/L)].

There are three lipoprotein classes carrying apolipoprotein B (ApoB): VLDL, LDL, and Lp(a). All these lipoproteins are removed by LA.

We measured ApoB in our patients in January 2021 and obtained the results shown in Fig. 23.2.

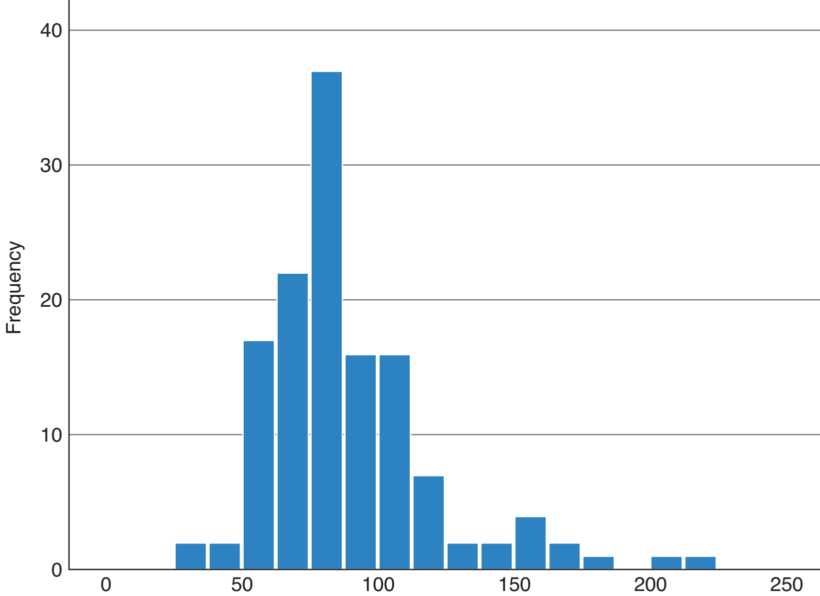
The median of ApoB is effectively acutely reduced by −70%. The mean ApoB concentration (between pre- and post-values) is 55 mg/dL.

In a recently published American statement on Lp(a), it was recommended to calculate the percentage of ApoB transported with the Lp(a) particles (Reyes-Soffer et al. 2022). Interestingly, in the HEART UK consensus statement, an expression of Lp(a) in molar units in order to appreciate its concentration relative to ApoB expressed in molar units is discussed (Cegla et al. 2019).

Our data before and after an LA session are depicted in Fig. 23.3.

The median is a little bit decreased. In Fig. 23.3b, it is shown that the percentage of ApoB contained in Lp(a) exceeding 20% is increased after the LA session comparing with the initial data.

The rebound of LDL and Lp(a) particle concentrations following LA allows the determination of fractional catabolic rate (FCR) and hence production rate (PR) during nonsteady-state conditions (Ma et al. 2019). The FCR of Lp(a) was significantly lower than that of LDL-ApoB, implying that different metabolic pathways are involved in the catabolism of these lipoproteins, with no significant differences in the corresponding PR.



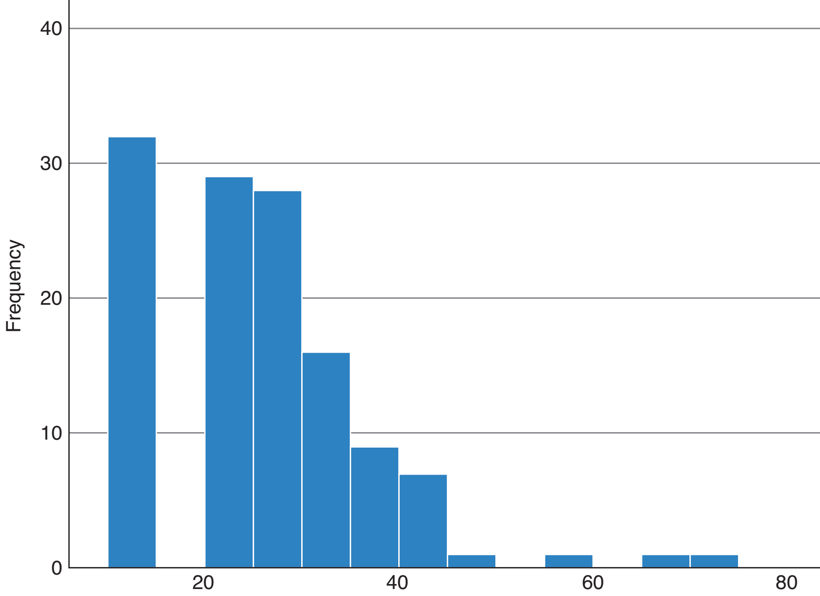
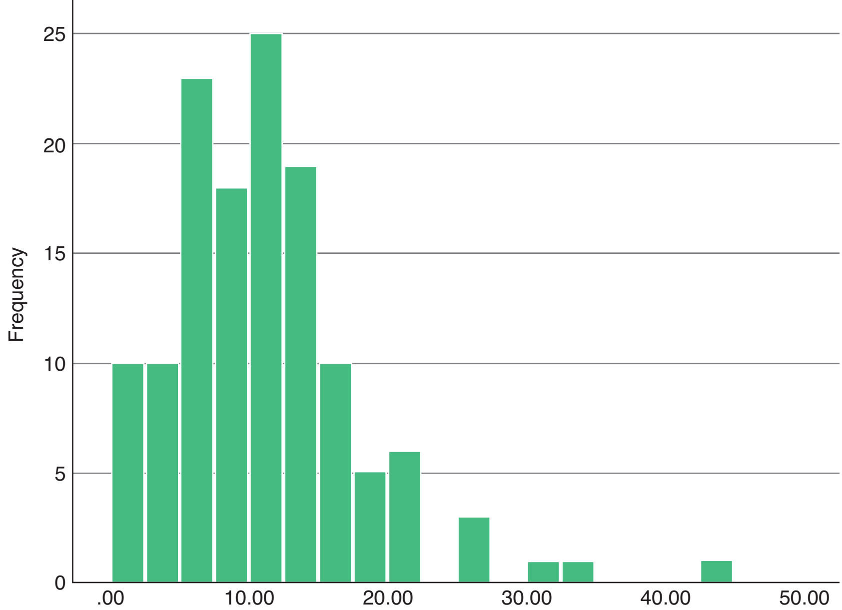


Fig. 23.2 ApoB concentrations (mg/dL) before (**a**) and after (**b**) one LA session. (**a**) Median 80 mg/dL (IQR 68/98 mg/dL). (**b**) Median 24 mg/dL (IQR 10/30 mg/dL)



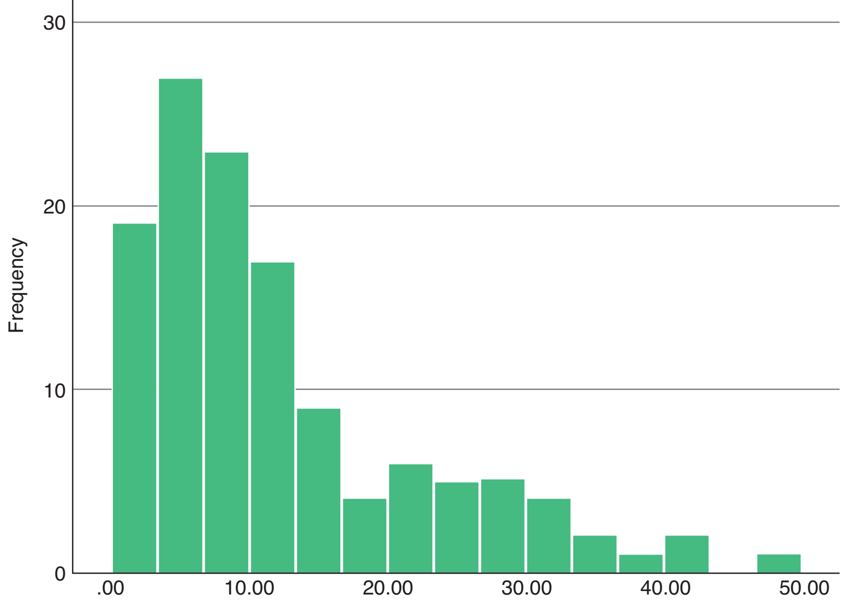


Fig. 23.3 Percentage of ApoB transported with Lp(a) particles before (**a**) and after (**b**) an LA session. (**a**) Median 11.3% (IQR 6.9/14.6%). (**b**) Median 9.8% (IQR 5.0/18.0%)

Oxidized Phospholipids

Oxidized phospholipids (OxPL) are mainly transported by Lp(a) particles and play an important role in atherogenesis (Yeang et al. 2016; Tsimikas 2019).

A small study in 18 patients with familial hypercholesterolemia and with low (≈10 mg/dL; range 10–11 mg/dL), intermediate (≈50 mg/dL; range 30–61 mg/dL), or high (>100 mg/dL; range 78–128 mg/dL) Lp(a) levels was performed to check the effect of LA on OxPL-ApoB and OxPL-apolipoprotein(a) [Apo(a)] concentrations (Arai et al. 2012). Plasma OxPL-ApoB was not reduced in the low Lp(a) group, but the levels were very low and near the level of detection of this assay. There was a strong trend for acute reduction (48%) in OxPL-ApoB in the intermediate Lp(a) group, and there was a significant decline (62%) in the high Lp(a) group. OxPL-Apo(a) was significantly reduced in all groups.

Adverse Effects and Contraindications of LA

Blood is handled outside the body. Different LA methods are characterized by a differing extracorporeal blood volume (Julius 2016). Moreover, an anticoagulation with heparin and/or citrate is needed to prevent blood clotting in the system.

Major adverse effects are hypotension, bleeding, and hypocalcemia (citrate effect). These occur rarely and can easily be treated (Dittrich-Riediger et al. 2015; Heigl et al. 2015). Some patients may show an iron deficiency (blood is taken to check lipid concentrations before and after LA sessions, other parameters are measured for security reasons) which often is relieved by intravenous iron application. A venous access on both arms is needed. Usually, experienced medical staff handles puncturing very well. Some patients need fistulas or shunts (less than 10% at our center, up to 30% in centers where nephrologists are in charge). These procedures may cause additional problems (e.g., thrombotic occlusions).

An extreme fear of patients with regard to this may be a contraindication against LA.

The list of contraindications (Table 23.3) against LA is rather limited (Julius 2016).

Table 23.3 Contraindications against LA [according to (Julius 2016)]

• No accessible veins (no possibility to establish a fistula)
• Severe heart failure, malignant cardiac arrhythmias
• Therapy-resistant hypotension
• Lack of compliance
• Foreseeable very short life expectancy
• Severe physical or intellectual inability of a given patient
• Presence of a malignant tumor with poor prognosis
• Severe psychiatric disorder

The intake of oral anticoagulants, especially new oral anticoagulants, is no contraindication for LA. When the HELP method is used, warfarin therapy should be avoided, due to danger of bleeding, because this method has a high impact on coagulation factors.

Efficiency of LA on Cardiovascular Events (CVEs) in Patients with High Lp(a) Concentrations

Observational Studies

Up to now, only observational studies on the effects of LA on outcome data in patients with elevated Lp(a) concentrations were published. German authorities asked for a prospective randomized controlled study to be performed in 2008. Ethics committees did not approve this type of study.

Observational Studies

In 2009, a longitudinal, multicenter, cohort study was published by Jaeger et al. In 120 patients [with an initial Lp(a) level of about 118 mg/dL], who were treated extracorporeally for about 5 years, the mean annual rate of major adverse coronary events per patient was reduced from 1.056 to 0.144 (−87%) (Jaeger et al. 2009). The rate of myocardial infarction was decreased by −97%. In the Pro(a) Life study, a prospectively conducted multicenter study, 170 patients were included (Leebmann et al. 2013; Roeseler et al. 2016; Klingel et al. 2019). The initial Lp(a) concentration was 108 mg/dL; LA sessions acutely reduced Lp(a) by −68%. When comparing with the situation before the start of LA therapy, major coronary adverse events declined by −78%; this finding remained stable up to the end of the 5-year follow-up.

Our Own (Dresden) Experience

The Dresden Center for Extracorporeal Therapy was involved in both studies. Moreover, we were the first to report that LA is more efficient with respect to the reduction of CVEs in patients whose Lp(a) levels are elevated when comparing with patients with normal or non-detectable Lp(a) (von Dryander et al. 2013; Schatz et al. 2017). This difference had been confirmed by another group (Heigl et al. 2015) and also in the German Lipoprotein Apheresis Registry (see below).

Moreover, we compared patients who developed CVEs while being treated with LA (n = 48) to those who did not suffer from CVEs (n = 60) (Julius et al. 2020). Both groups were on extracorporeal therapy for years already, for a mean period of

5–6 years. Interestingly, no difference with respect to lipid concentrations, including Lp(a), was observed. But two factors had a significant impact on the occurrence of new CVEs during LA: (1) older age at the start of the extracorporeal therapy (Patients with events were about 5 years older, in the mean 60 years.) and (2) a higher number of CVEs before the initiation of LA—a positive correlation between this number and the number of CVEs during LA was calculated.

Italian Retrospective Multicenter LA Study in Patients with Elevated Lp(a) and Coronary Artery Disease

Twenty-three patients with Lp(a) levels above 60 mg/dL and a pre-apheresis LDL-C <100 mg/dL on maximally tolerated lipid-lowering therapy were included (Bigazzi et al. 2018). They were treated with LA for several years (median 7, interquartile range 3–9 years) by heparin-induced LDL precipitation apheresis (16/23), dextran-sulfate (4/23), cascade filtration (2/23), and immunoadsorption (1/23). The time lapse between first cardiovascular event and beginning of apheresis was 6 years (interquartile range 1–12 years). The rates of adverse cardiovascular events were reduced by 74% when comparing the situation before and after the LA treatment inception.

Study in Patients with Elevated Lp(a) and Peripheral Artery Disease (PAD)

Ten patients with severe PAD and isolated Lp(a)-HLP who recently underwent revascularization (index procedure) were included (Poller et al. 2017). When comparing the situation before LA with the results after 12 months, the pain level, ankle-brachial index (ABI), transcutaneous oxygen pressure ($tcpO_2$), and walking distance all improved. Importantly, the frequency of revascularization procedures was strongly decreased under LA. All patients combined underwent 35 revascularizations within the 12 months prior to the index procedure (mean interval between two revascularizations: 104.3 days). Since the index procedure, only one revascularization was necessary within 79 patient-months under LA (mean interval: 2404.5 days, $p < 0.001$).

American Single-Center, Retrospective Cohort Study in Patients with High Lp(a) Levels

Fourteen patients with cardiovascular disease with elevated Lp(a) and near-normal LDL-C were treated with LA over a mean treatment period of 48 months (range 8–105 months) (Moriarty et al. 2019). The authors describe a 94% reduction in major adverse cardiovascular events.

German Lipoprotein Apheresis Registry

This registry has existed since 2011. In the annual report for 2020, data on 1111 patients (from 44 LA centers, 6791 LA sessions) have been documented (Schettler et al. 2020). Following the suggestion from Dresden, three hyperlipoproteinemia (HLP) groups have been defined (based on the initial lipid values): (a) with isolated elevation of LDL-C [Lp(a) level <60 mg/dL or <120 nmol/L or not detectable, $n = 180$], (b) with isolated elevation of Lp(a) [Lp(a) ≥60 mg/dL or ≥120 nmol/L and LDL-C <2.6 mmol/L, $n = 500$), and (c) with combined elevation of both LDL-C and Lp(a) (using the abovementioned criteria, $n = 228$). The latter group is totally neglected in the officially published data.

The following mean acute reduction rates were reported for the LA sessions:

LDL-C—69%
Lp(a)—73%

The mean Lp(a) concentrations (the mean levels are a surrogate parameter for the interval mean values; formula: mean = ½ × (pre value + post value); levels have been separately reported according to the dimension provided by the lab): Group B—54.20 mg/dL and 98.60 nmol/L, respectively; Group C—61.50 mg/dL and 104.75 nmol/L, respectively.

Mean LDL-C levels ranged between 2.00 mmol/L (Group A) and 1.59 mmol/L (Group C). LDL-C levels for Group B are not given in detail.

CVEs have been subdivided into MACE [major adverse cardiac events: acute coronary syndrome (unstable angina pectoris, NSTEMI, STEMI), coronary intervention/surgery (PTCA, stent, CABG)] and MANCE [major adverse noncardiac events: arterial occlusive disease (AOD) at noncoronary arteries with occlusion or necessity for intervention/operation (PTA, stent, bypass, amputation), AOD of brain arteries with TIA/stroke (CAOD), AOD of aorta thoracalis or aorta abdominalis including visceral vessels and renal arteries, peripheral arterial occlusive disease (PAOD)] (Table 23.4).

Follow-up data are contained in the annual report of the registry up to 7 years under LA; low CVE rates were constantly seen throughout these years.

As an example, the graphs for HLP Group B are depicted (Fig. 23.4; with permission of the Lipid-League).

Table 23.4 Percent reductions of MACE (major adverse cardiac events) and of MANCE (major adverse noncardiac events) in the HLP Groups A, B, and C comparing the 2 years before the start of LA with those during the first 2 years under LA (Schettler et al. 2020)

HLP group	Reduction of MACE (%)	Reduction of MANCE (%)
A	61	25
B	83	64
C	72	65

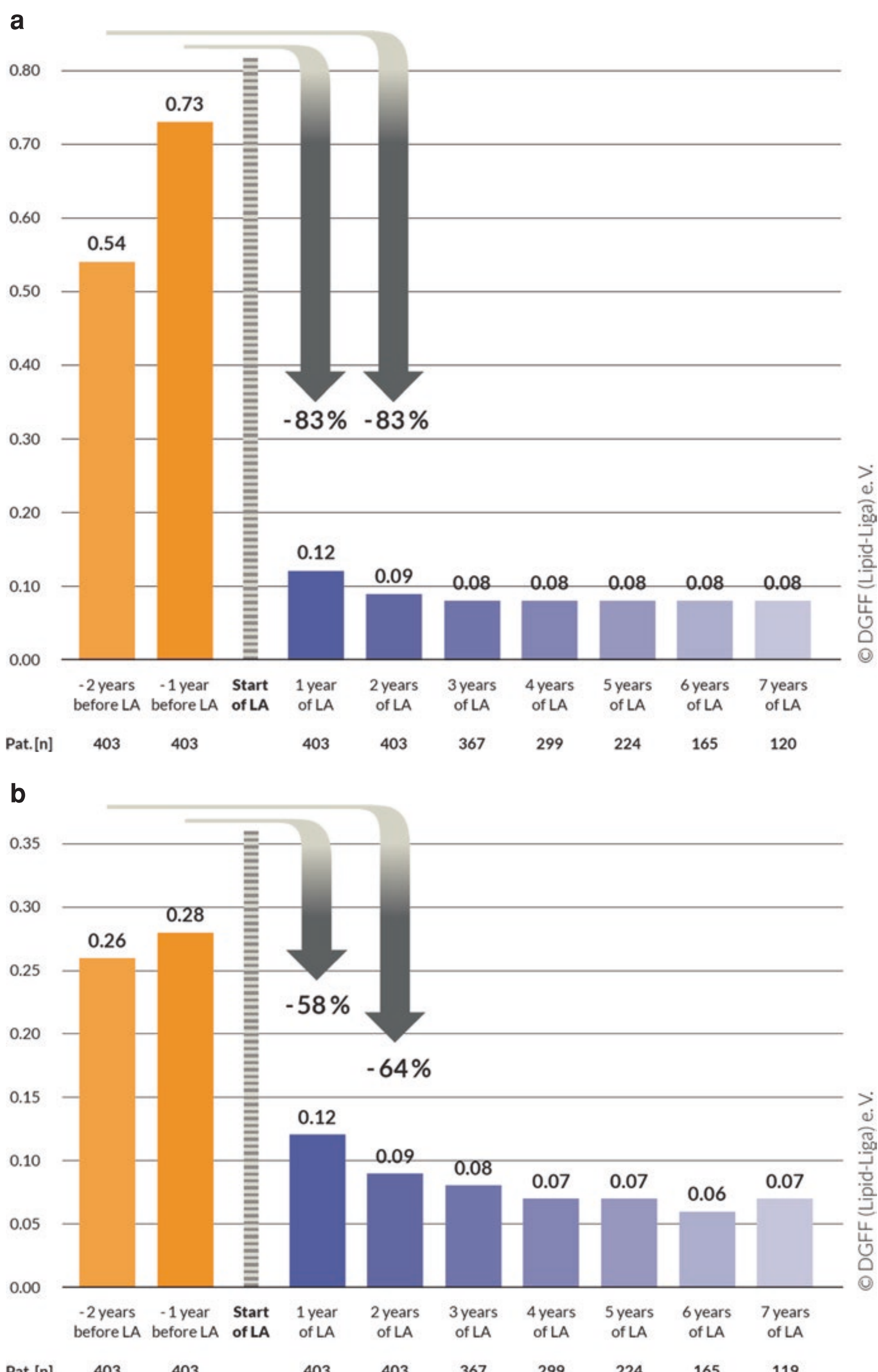


Fig. 23.4 MACE (**a**) and MANCE rates (**b**) in HLP Group B (with permission of Lipid-League). (**a**) MACE rate (per patient and year) in HLP Group B. (**b**) MANCE rate (per patient and year) in HLP Group B

Many patients had suffered from CVEs in the years anteceding the years included in these graphs.

These data underline the already previously reported higher efficiency of LA with respect to the reduction rate of CVEs in patients with elevated Lp(a) when compared to patients without this feature.

Russian-Specific Columns Against Lp(a) [Lp(a) Lipopak® Adsorption Columns]

The commonly available LA systems decrease both LDL-C and Lp(a) concentrations. The Russian POCARD Ltd. company offers antibody-coated columns which specifically decrease Lp(a) only (Pokrovsky et al. 2017; Safarova et al. 2013). A slight decrease in LDL-C does not represent the removal of LDL but only the removal of cholesterol in Lp(a) particles. In a prospectively carried out angiographic study over 18 months, it could be shown that a weekly Lp(a) reduction was associated with decrease in the mean percent diameter stenosis by 5.05% and increase in minimal lumen diameter by 14%; mean total atheroma volume was reduced by 4.60 mm^3 ($p < 0.05$ for all). These data were compared to those seen in the control group which was treated with atorvastatin only. This small study points to the effectiveness of a specific elimination of Lp(a) as a tool to combat atherosclerotic lesions. Unfortunately, these specific columns are not used in a large scale anywhere.

MultiSELECt Study

MultiSELECt, a prospective European multicenter study on the effect of Lp(a) elimination by LA on cardiovascular outcomes, was designed to directly compare subjects with significantly elevated Lp(a) approved for LA subsequently undergoing apheresis treatment versus a continuation of maximal medical therapy (Hohenstein et al. 2017). The study aims at establishing matched pairs; it is still ongoing (NCT02791802).

Other Effects of LA in Patients with High Lp(a): A Study with Sham Control

A British group conducted a prospective randomized, sham-controlled, single-blinded, crossover study involving 20 patients with refractory angina and elevated Lp(a) >500 mg/L and LDL-C <4 mmol/L (Khan et al. 2017). Patients were randomized to a treatment arm with weekly LA for 3 months (12 sessions) or a control

group with placebo “sham” sessions weekly for 3 months. Treatments were performed using the DX21 DHP (Direct Hemo Perfusion) Lipoprotein Apheresis machine (Kaneka Corporation, Osaka, Japan) with the Liposorber DL-75 column, which uses dextran sulfate to covalently bind ApoB-containing lipoproteins. Baseline tests were repeated after treatment periods for both groups. After a 1-month washout period, patients crossed over to the opposite treatment arm.

Patients underwent cardiovascular magnetic resonance imaging at baseline assessing quantitative first-pass stress/rest perfusion and assessment of carotid atherosclerosis with measurement of total carotid wall volume. Patients had exercise capacity tested using the Six Minute Walk test (6MWT) and assessment of their angina symptoms using the Seattle Angina Questionnaire (SAQ) and quality of life (QoL) with the SF-36 questionnaire.

The results indicate that an improvement in myocardial perfusion rate was primarily driven by improvements in stress perfusion, with insignificant change in rest perfusion. In terms of secondary endpoints, improvements with apheresis compared with sham also occurred in carotid atherosclerotic burden as assessed by total carotid wall volume ($p < 0.001$), exercise capacity measured by the 6MWT ($p = 0.001$), four of five domains of the SAQ (all $p < 0.02$), and quality of life physical component summary assessed by the SF-36 survey ($p = 0.001$).

Selected Case Reports

Some patients with elevated Lp(a) levels develop CVEs in several arterial regions. We report two patients who were treated with LA at our center. Both suffered from new CVEs despite being treated extracorporeally and were switched to two LA sessions per week (Figs. 23.4 and 23.5).

Despite the intensive therapeutic regimen starting in 2018, the patient underwent new interventions of her carotids, leg, and visceral arteries. Before being referred to our center, she was smoking and it was not easy to persuade her to stop this habit.

In Fig. 23.4, the lipid data are also shown. Lp(a) was effectively reduced, but in all these years, LDL-C remained above the requested level (1.0 mmol/L). That is why we started inclisiran in 04/2021. Since then, no new CVEs were observed. Her actual lipid concentrations are as follows (before/after an LA session in 2022/02): LDL-C 0.66/0.14 mmol/L, Lp(a) 153/26 nmol/L, HDL cholesterol 1.43/1.12 mmol/L, and triglycerides 1.68/0.56 mmol/L. Now LDL-C is optimal; Lp(a) level is still high.

Another patient suffered from severe atherosclerosis of his coronaries (Fig. 23.6).

At the age of 39 years, he had an acute myocardial infarction. His Lp(a) concentration was found to be extremely high (≈593 nmol/L) only in 2006 (23 years after his MI). He was also suffering from an increasing statin intolerance. From 1994 to

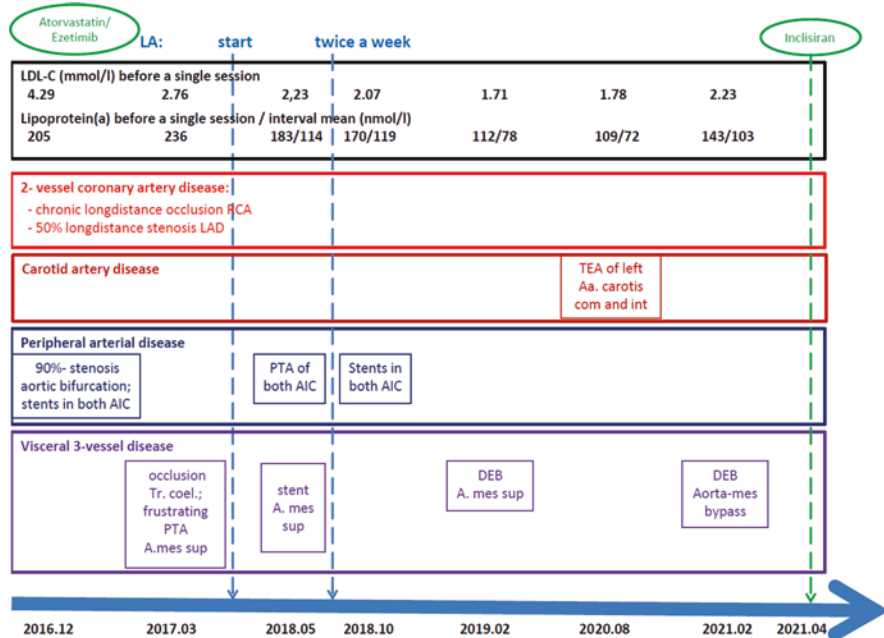


Fig. 23.5 A 56-year-old female patient with atherosclerotic affections of all vessel territories; LA was started in 2017, switched to two sessions per week in 10/2018 (patient agreed that her data could be included into this manuscript)

2011, he needed several interventions at his coronaries—in 1998, a fourfold coronary bypass was performed. After 4 years of weekly LA therapy, he came to the sessions twice per week. Nevertheless, he needed further interventions, though his Lp(a) levels were clearly reduced. On the background of LDL-C concentrations of more than 2 mmol/L before the LA sessions, we initiated an evolocumab injection therapy in 2019. Since then, no new CVEs occurred.

His current lipid levels are as follows (before/after an LA session in 10/2022): LDL-C 2.09/0.45 mmol/L, Lp(a) 215/31 nmol/L, HDL cholesterol 1.34/1.19 mmol/L, and triglycerides 2.48/0.45 mmol/L.

At our department, we treat several patients whose indication for an LA therapy was an elevation of Lp(a) which was likely, after the exclusion of cardiac reasons, responsible for strokes (Table 23.5). In the literature, this association had been described (Nave and von Eckardstein 2019; Arnold et al. 2021). Lp(a) seems first of all to be responsible for large artery atherosclerosis stroke. In a Russian paper, it was reported that in logistic regression analysis adjusted for age, sex, hypertension, type 2 diabetes, smoking, and Lp(a) concentration, the hyperlipoproteinemia(a) was associated with ischemic stroke and isolated stenotic carotid atherosclerosis

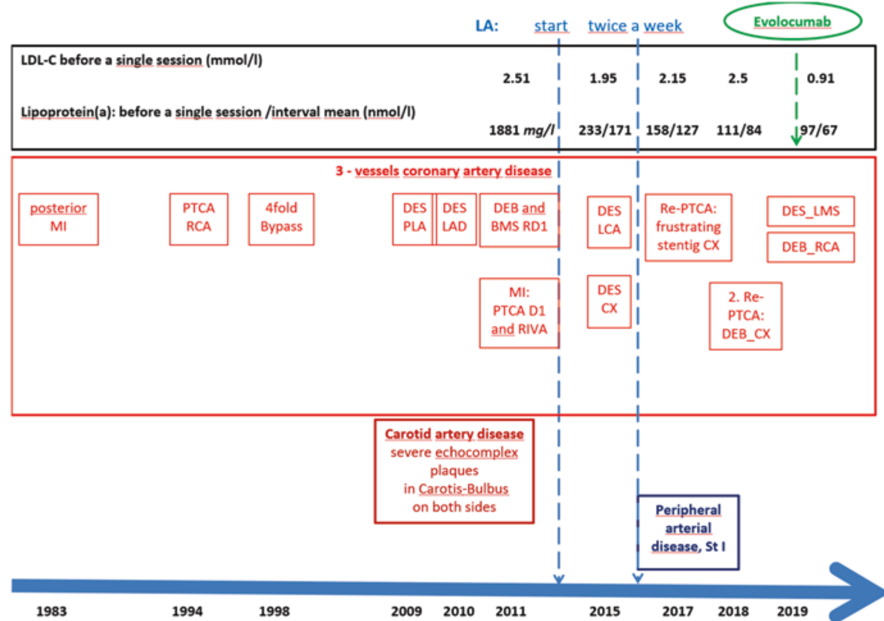


Fig. 23.6 A 77-year-old male patient with multiple events at his coronary arteries; LA was started in 2012 and switched to two LA sessions per week in 2016 (patient agreed that his data could be included into this manuscript)

(Tmoyan et al. 2020). In the group with severe carotid atherosclerosis, 16 patients (24%) had ischemic stroke. Lp(a) concentration in these patients was higher 36 [20; 59] mg/dL than in the patients with isolated carotid atherosclerosis without stroke 15 [7; 54] mg/dL ($p = 0.04$).

All patients listed in Table 23.5 had an elevation of Lp(a) and a (mostly) modest increase of LDL-C. Only in two patients atherosclerotic lesions of their carotids were documented. In one patient, coronary atherosclerosis was observed. One patient does not take a statin (statin intolerance, he started evolocumab in 2019).

Our patients did not develop any further strokes after they started to be treated with LA, though Lp(a) interval mean values were not optimal.

In the literature, a genetically lowered Lp(a) concentration predicted a decreased risk of stroke (Kamstrup 2021). An elevated Lp(a) level was associated with unfavorable functional outcomes in patients with ischemic stroke (Jiang et al. 2021). Thus we are convinced that the lowering of Lp(a) levels by LA is a beneficial contribution to the further follow-up situation of stroke patients.

Table 23.5 Male patients who are treated with LA because of an elevation of Lp(a) and following cerebrovascular events (patients agreed that their data could be included into this manuscript)

ID	Born	Stroke events		Atherosclerosis	ApoE	LA start	LDL-C (mmol/L)		Lipoprotein(a) (nmol/L)		Statins: name/ daily dose (mg)
		Year	Localization				Before LA	Actual	Before LA	Actual/ interval mean	
RB	1967	2019 nk	Left A. Cerebri Media Old lacunar infarctions in white matter	Mild in Aa. carotis Subcortical arteriosclerotic encephalopathy One-vessel-CAD, no interventions	3/3	08/04/20	2.0	1.7	363	321/236	A/80
MG	1980	2017	Caput nuclei caudate, on the left side	No	3/3	02/27/19	1.56	1.46	125	138/90	S/30
HK	1962	2003 2007 2008 2009 2009	Cerebellum Left A.cer media left A.cer ant Left A.cer ant parietal operculum	TEA in the left A. car int in 2007 Actual—severe plaques Aa. carotis without relevant stenosis	4/3	03/23/20	1.41	1.43	137	95/62	No
F-PK	1968	2020	Cerebellum	No	3/3	08/25/20	4.76	1.83	428	287/214	A/60
HS	1986	2018	Left A.cer media	No	4/3	05/16/19	1.94	1.39	120	121/56	A/40
U-OS	1978	nk 2020	Cerebellum: small old defect Vertebral-basilar TIA	No	Nk	01/07/21	2.5	1.22	346	227/151	A/60
JW	1980	nk 2017	Cerebellum: old lacunar defect Right basal ganglia	No	3/3	01/28/20	3.21	2.3	152	122/87	R/10

Actions Which Should Be Taken in Patients Who Develop Cardiovascular Events (CVEs) Despite Being Treated with LA

Of course, these patients should be continuously advised to follow the rules of a healthy lifestyle (no smoking, diet, regular intake of drugs). The permanent contact with physicians and the medical staff offers opportunities to regularly discuss these aspects.

In very few patients with an extremely high cardiovascular risk (see selected cases above), we decided to perform two LA sessions per week.

In patients whose LDL-C concentrations remained clearly above the internationally recommended target (1.4 mmol/L in high-risk patients, 1.0 mmol/L in those with repeated CVEs) (Mach et al. 2019) despite taking statins and ezetimibe (when tolerated), we started an injection therapy with PCSK9 antibodies (evolocumab, alirocumab). Presession Lp(a) levels were decreased between 0 and 44% after 12 weeks injecting these drugs (Julius et al. 2019). In the last months, we also initiated a therapy with inclisiran.

As shown in the chapter on selected cases, the addition of injections of PCSK9 inhibitors stopped the progression of CVEs in extremely high-risk patients.

Unresolved Problems

Though LA therapy is performed, especially in Germany, for more than 30 years now, some questions still remain unanswered.

Which LA Method Is the Best?

At the Dresden Center for Extracorporeal Therapy, we have a long-lasting experience with six different LA methods (Julius 2016). When we observe an insufficient acute decrease of Lp(a) in a given patient, we try to optimize the situation, for example, by increasing the treated plasma/blood volume. If the result is not satisfying, we usually switch to another system.

In a paper which appeared in 2013, we recommended that each apheresis center should work with more than one LA system (Julius et al. 2013). The calculations presented in this paper are based on laboratory data measured at the last three available apheresis sessions before switching to another method and at the end of the observation period, respectively. With respect to the reduction of LDL-C, DALI and Liposorber D appeared to be the most effective LA methods, for reduction of Lp(a), Liposorber D. In any comparisons between the LA methods in the following years, we did no longer observe any differences with respect to lipid lowering data—in other words, we are treating our patients quite effectively.

Other differences between LA systems have been described, for example, for proteins, PCSK9 levels, and coagulation factors (Julius et al. 2002, 2015a, b). The significance of these differences for the prognosis of the patients remains still to be clarified.

Moreover, additional pleiotropic effects of LA (removal of C-reactive protein, complement, of apolipoprotein CIII, TNF-α, interleukin 6, and adhesion molecules like ICAM-1 or VCAM-1) may have an impact on the course of atherosclerotic lesions (Waldmann and Parhofer 2016; Makino et al. 2019). LA leads to vascular tone reduction, reduced thrombogenesis, increased neo-angiogenesis, and importantly plaque stabilization (Poller et al. 2017). No data comparing different LA methods with regard to these parameters have been published.

How Low Should Lp(a) Be to Effectively Prevent New CVEs During LA Therapy

For LDL-C in the last years, target values have been defined with the aim to effectively reduce the cardiovascular risk. The major message is "the lower the better."

In the absence of randomized, controlled trial data demonstrating reduced cardiovascular risk with reduction in Lp(a), no such targets have been proclaimed for this parameter.

Usually, in lab reports, a normal range for Lp(a) is given below 30 mg/dL (about 75 nmol/L). It has been discussed in a paper by Boffa et al. that lowering Lp(a) below this threshold would ameliorate the atherogenic risk (Boffa et al. 2018).

We think that in order to obtain an optimal effect of LA therapy with respect to CVEs, interval mean values should be normalized [probably below 30 mg/dL (about 75 nmol/L)]. As shown in Fig. 23.1, the reality is far from this request. In only 21% of our patients, this goal was reached. About half of them had an interval mean Lp(a) concentration higher than 120 nmol/L. Though as a matter of fact, we did not see a relationship between these concentrations and the incidence of CVEs during LA therapy (Julius et al. 2020). And in the Russian prospective study, using specific anti-Lp(a) columns, with coronary angiography, a beneficial effect on coronary atherosclerosis was observed, though the mean interval value in the apheresis group was 73 mg/dL (about 175 nmol/L) (Pokrovsky et al. 2020).

In the literature, two papers suggested, based on data with Mendelian randomization, that a decrease of Lp(a) by about 100 mg/dL (about 240 nmol/L) (Burgess et al. 2018) or 65 mg/dL (about 156 nmol/L) (Lamina et al. 2019) will induce a similar reduction of CVEs as a decrease of 1 mmol/L of LDL-C, for example, by about 22%. Populations included into these meta-analyses had much lower median Lp(a) concentrations (approximately 30 nmol/L, maximally in one study 104 nmol/L) than those who are usually treated extracorporeally. Moreover, both studies were population based. In contrast, a Danish group looked at patients with a history of cardiovascular disease who were followed after their initial event (Madsen et al. 2020). The authors calculated that plasma Lp(a) should be lowered by 50

(about 120 nmol/L) and 99 mg/dL (about 240 nmol/L) for 5 years to achieve 20% and 40% MACE risk reduction in secondary prevention. Accordingly, for a 22% MACE reduction, a reduction of Lp(a) by 55 mg/dL (about 132 nmol/L) would be required.

From the viewpoint of LA data, these publications are not conclusive at all.

In the HPS2-THRIVE Study, niacin laropiprant reduced mean Lp(a) by 12 nmol/L overall and by 34 nmol/L in the top quintile by baseline Lp(a) level ≥128 nmol/L (Parish et al. 2018). The authors write that estimates from genetic studies suggest that these Lp(a) reductions during the short term of the trial might yield proportional reductions in coronary risk of ≈2% overall and 6% in the top quintile by Lp(a) levels.

In studies using PCSK9 antibodies (evolocumab, alirocumab), a small decrease of Lp(a) concentrations was seen (Kronenberg 2022; Julius et al. 2019). When excluding the impact of the reduction of LDL-C on outcome data by mathematical modeling, the decrease of Lp(a) was effective with respect to a certain lowering of CVEs when compared with the placebo groups.

A comparison between PCSK9 antibodies and pelacarsen [an antisense oligonucleotide against Apo(a)] showed an interesting difference: Pelacarsen reduced Lp(a) by 47% and as a consequence the pro-inflammatory gene expression in monocytes of cardiovascular disease patients with elevated Lp(a), which coincided with a functional reduction in transendothelial migration capacity of monocytes ex vivo (Stiekema et al. 2020). In contrast, PCSK9 antibody treatment lowered Lp(a) by 16% and did not alter transcriptome nor functional properties of monocytes, despite an additional reduction of 65% in low-density lipoprotein cholesterol (LDL-C). The effect of Lp(a) lowering by LA is in the same range as described for pelacarsen in this manuscript.

Should We Calculate "True" LDL-C?

When LDL-C is measured, both LDL and Lp(a) particles are included (Yeang et al. 2015). In order to calculate the LDL-C mass transported with LDL, the following steps are required: (1) The Lp(a) mass should be in mg/dL—we measure Lp(a) in nmol/L—the conversion into the Lp(a) mass is not correct (and no longer recommended). (2) The estimated percentage of LDL-C in the Lp(a) particles usually is set to be 30%. Data have shown that this percentage may be variable interindividually. (3) Due to these problems, in some patients, negative "true" LDL-C levels are seen. The British colleagues do not recommend to calculate "true" LDL-C because (1) it is not validated with isoform-independent assays in treated and untreated patients, (2) it is not validated in large epidemiological studies for cardiovascular risk prediction or in RCTs of lipid-lowering therapies, and (3) it is not in clinical use (Cegla et al. 2019). Recently, a novel method for quantification of Lp(a) cholesterol had been suggested (Yeang et al. 2021). This problem is relevant for patients who are treated with LA and with PCSK9 inhibitors.

LA in Children with Ischemic Stroke and Patients on Hemodialysis with High Lp(a) Levels

In children, a highly elevated Lp(a) concentration may be associated with an increased risk for ischemic stroke (deVeber et al. 2019). The arterial ischemic stroke-free survival in children with elevated Lp(a) was lower compared with that in the remaining children with normal Lp(a) levels. The authors do not mention LA as a therapeutic option.

Higher Lp(a) values and LDL-unbound Apo(a) particles were found in patients with end-stage renal disease; their LDL has different chemical and structural properties as compared to control (Trenkwalder et al. 1997). Apheresis would be an optimal tool to remove all these atherogenic lipoproteins. In reality, a few patients who are treated with hemodialysis due to renal insufficiency have started an LA treatment on the background of severe atherosclerotic complications.

No outcome data for the combination of hemodialysis and LA are available.

Future of LA in Patients with High Lp(a) Levels

Pros and Cons of LA

LA allows the treatment of high-risk patients with elevated Lp(a) levels who have suffered from CVEs. It is tolerated very well. In patients with familial hypercholesterolemia, which is not seldom associated with elevated Lp(a) levels, tendon xanthomas usually disappear under a year-long LA therapy. This points to the fact that the body cholesterol pool is diminished.

The extracorporeal removal of Lp(a) particles significantly decreases their concentration in blood, especially in the days immediately after LA sessions. The increase of Lp(a) thereafter makes it necessary to perform LA sessions weekly. In some countries, a 2-week interval is the prevailing therapeutic approach, mainly because of financial problems.

Interval mean values reflect approximately the averaged Lp(a) level in the days between LA sessions and in this way the atherogenic burden. With the available LA methods, it is possible to reach optimal Lp(a) concentrations (<75 nmol/L) only in a small part of the patients. But extremely high Lp(a) levels which confer a very high atherogenic risk can be reduced a lot.

In LA patients, all other risk factors (hypertension, diabetes mellitus, hyperuricemia, smoking habit, hypothyroidism, obesity) should be optimized.

The usual way to describe the effect of LA therapy on outcome data—by comparing the incidences before the start of the extracorporeal treatment with those during this treatment—has been criticized (Waldmann and Parhofer 2016). It should be noted that the observational studies suffer from potential confounding due to the selection bias for survivors inherent in their design, as well as the lack of the ability

to rule out the effect of apheresis on other drivers of events such as fibrinogen (Boffa et al. 2018). Hopefully, the MultiSELECt study will offer a clarification of this dispute. Clearly, placebo-controlled apheresis studies are not feasible. The British study which had an individual sham control lasted only a few months.

Under extracorporeal therapy, some patients will develop new CVEs. We did not detect any difference in lipid concentrations, including Lp(a), before or after LA sessions or in interval mean values between patients with or without CVEs during LA therapy (Julius et al. 2020). According to our data, older age at the start of the LA therapy and a higher number of CVEs before LA started playing a role. Both these aspects point to the fact that atherosclerosis has progressed in patients who suffer from CVEs during the extracorporeal therapy.

Thus, in order to be on the safe side, an LA therapy should not be initiated too late. But on the other hand, LA may be lifesaving in high-risk patients. The number of patients who die when they are undergoing LA therapy is rather low; no reliable data on these numbers are available.

In general, an LA therapy should not be started in patients who are (biologically) older than 70 years. On the other hand, LA is a lifelong treatment and should not be discontinued even in very old patients who started LA years ago. The diagnosis of a malignant tumor, a severe cardiac insufficiency, or a poor compliance may be reasons for stopping the extracorporeal therapy.

In the British sham-controlled apheresis study, a regression of atherosclerosis was seen at the neck vessels. In some angiographic studies, a certain percentage of regression at the coronary arteries was found in LA-treated patients. In our experience, we are already happy when in a given patient a nonprogression of the lesions (no new stenoses, no new CVEs) is observed. Of course, patients should regularly be checked by a cardiologist and/or angiologist.

LA is expensive and time-consuming (2–3 h) and needs the work of a qualified staff. But by avoiding new CVEs, money can be saved in the long run. In Germany, nephrologists may apply for the permission to perform LA. Taking into attention the fact that LA can be optimally performed only in centers with sufficient experience in this field, the number of patients at each center should not be too low (probably not less than ten patients, at least two LA systems should be offered).

Diet, Statins, Ezetimibe, Bempedoic Acid, PCSK9 Inhibitors, and Evinacumab

Each patient who is taking lipid-lowering drugs should be advised to adhere to a healthy diet as well. This rule is also valid for patients with high Lp(a) concentrations though nutrition does not exert any effect on Lp(a). In patients with familial hypercholesterolemia, the effectiveness of diet on LDL-C is rather limited. Despite these restrictions, in Germany, insurance companies demand that a consultation about diet should be documented; this also concerns all LA patients.

If in a given patient with elevated Lp(a) concentration the LDL-C level exceeds the internationally accepted targets (1.4 mmol/L for high-risk patients, 1.0 mmol/L for patients with repeated CVEs) (Mach et al. 2019), usually a statin therapy should be started. More effective statins (atorvastatin, rosuvastatin) are to be preferred. When the effect is not satisfactory, ezetimibe can be added. Bempedoic acid can be administered either together with a statin (in order to improve LDL-C) or instead of a statin when the latter is not tolerated. Ezetimibe can be continued in these cases. When the result seen after the introduction of these first steps is not optimal, after several months, the indication to use PCSK9 inhibitors is given.

All these measures are the prerequisite before an LA is allowed to be started, at least in Germany.

An LA therapy may be commenced in the following LDL-C ranges in patients with Lp(a) concentrations exceeding 60 mg/dL or 120 nmol/L:

1. The LDL-C target has been reached. That is the purpose of the official regulations.
2. The LDL-C target was not reached despite the patient regularly took the lipid-lowering drugs. This indication is not officially covered by the existing rules, but this situation is not a very rare one.
3. Patients experience a drug intolerance—this may be the case with statins or PCSK9 inhibitors, very seldom with ezetimibe. LDL-C is still too high.

Of course, a progression of atherosclerosis has to have been documented (repeated CVEs or shown by imaging techniques). Exceptions from this demand are made in very young patients (aged under 40 years) with extremely high Lp(a) levels who survived a life-threatening acute myocardial infarction and who have a positive family history for CVEs among first-degree relatives in younger ages.

During the LA therapy, the administration of lipid-lowering therapy should always be continued. Some adjustment of doses may be necessary depending on the measured LDL-C level.

In our hands, the addition of PCSK9 inhibitors to LA procedures may further improve LDL-C and—at least in a majority of patients—Lp(a) concentrations. The combined treatment with PCSK9 monoclonal antibodies and apheresis may be preferable in certain hypercholesterolemic patients with high Lp(a), because of the combined benefits of both approaches in lowering LDL-C, triglyceride-rich lipoproteins, inflammation, hemorheology, and Lp(a) (Ruscica et al. 2019).

We saw the end of a series of cardiovascular interventions in high-risk patients after the start of this intensive injection therapy. In some patients, we then switched to a biweekly LA regimen—provided the cardiovascular situation remains stable.

Evinacumab, a fully human monoclonal antibody against angiopoietin-like 3, is a new drug which can be applied in therapy-resistant hypercholesterolemia (Rosenson et al. 2020). But this drug does not decrease Lp(a). In an ApoE*3-Leiden CETP mouse model, a triple therapy with atorvastatin, alirocumab, and evinacumab has been successfully performed (Pouwer et al. 2020). The future role of intravenously infused evinacumab in the daily routine has still to be defined.

Pleiotropic effects of LA differ essentially from those described for lipid-lowering drugs. The combination of drug and LA therapy promises to obtain the best clinical results.

Inhibitors of Apo(a) Synthesis

The antisense oligonucleotide AKCEA-APO(a)-LRx (pelacarsen) effectively reduces Lp(a) levels (up to 80%) by impairing the synthesis of Apo(a) (Tsimikas et al. 2020). Since 2020, a prospective, placebo-controlled Phase III HORIZON study (ClinicalTrials.gov Identifier: NCT04023552) is ongoing. The tolerability of pelacarsen is described to be very good. A special focus in this study is on obtaining low LDL-C values, even PCSK9 inhibitors are allowed. It will be interesting to see what will be the effect of pelacarsen on outcome data.

Moreover, two other companies are currently testing small interfering RNA drugs against Apo(a) (Amgen: Olpasiran (AMG 890); ClinicalTrials.gov Identifier: NCT04270760) and Silence Therapeutics plc (SLN360; ClinicalTrials.gov Identifier: NCT04606602). Olpasiran was already used in a phase 1 dose escalation trial (Koren et al. 2022).

These drugs will be a competitor to LA with regard to the indication "isolated elevation of Lp(a)," provided the outcome data of the HORIZON study will be convincing.

At present, two problems with these drugs are evident: (1) They will be r expensive. (2) They do not show any effect on LDL-C—drugs to lower LDL-C are not always effective enough to reach target levels; they are not well tolerated in a substantial number of patients.

It can be supposed that some patients with an extremely high atherogenic risk will still need the extracorporeal therapy in order to save their life. The commonly used LA procedures decrease both Lp(a) and LDL-C and exert some beneficial pleiotropic effects.

Ideally, a study comparing prospectively Apo(a) synthesis inhibitors with LA with respect to the occurrence of CVEs could answer the question what will be the best way to treat high-risk patients with elevated Lp(a).

Conclusions

LA is at present the only accepted therapy to decrease highly elevated Lp(a) concentrations in high-risk patients with the aim to revert a progressive course of Lp(a)-associated cardiovascular disease to a stable course and to prevent future CVEs. Most probably, pleiotropic (anti-inflammatory, antithrombotic, rheologic) effects exert an additional benefit. Up to now only observational studies documented a high efficiency of LA with respect to reduction of the incidence of CVEs. LA requires a

high qualification of the medical staff, is time-consuming, and is expensive but is associated with good tolerability.

LA therapy should only be initiated when dietary efforts and all conventional lipid-lowering drugs (when tolerated) have been brought into play and patients experienced CVEs. When Lp(a) will be measured more often in high-risk patients (which can already be observed today), the indication of an LA therapy will have to be considered more often.

Unfortunately, at present, the role of LA for reduction of Lp(a) is not accepted everywhere. In a new review about elevated Lp(a) levels in persons with familial hypercholesterolemia—this association is described to be highly atherogenic—the Danish scientists do not mention apheresis at all (Langsted and Nordestgaard 2022). LA was shown to decrease both Lp(a) and LDL-C—this combined effect should be especially beneficial in patients who are resistant to the usual lipid-lowering therapy or who do not tolerate these drugs.

Drugs inhibiting the synthesis of Apo(a) will represent a competitor to LA for Lp(a) patients in the future. For these drugs, outcome data showing an advantage in comparison with LA will be required. A major advantage of these drugs is that Lp(a) levels remain permanently low, while on LA therapy, they are fluctuating. Nevertheless, some patients with an extremely high atherogenic risk (severe affection of several vessel territories on the background of a positive family history for early CVEs, progression despite an optimal therapy with lipid-lowering drugs) will still need extracorporeal therapy in order to survive in the future. This will especially be the case in patients who did not show a sufficient decrease of LDL-C concentrations.

Finally, there is still another important aspect which is cited here literally from Thompson and Parhofer (2019): "Patients treated by regular apheresis have the advantage of being seen by the same medical team on a very regular (weekly or biweekly) basis. This tight control and guidance improves compliance (generally speaking) and allows medical issues to be discussed regularly in a familiar setting. Although this effect is hard to quantify, it would be surprising if it did not also affect the cardiovascular event rate. Obviously, drug therapy gives the patient more "freedom" but maybe at the cost of less strict medical surveillance."

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Chapter 24
Elevated Lp(a): Why Should I Test For It, If I Cannot Treat It? A Patient's Perspective

Sandra Revill Tremulis

"How Can I Have Normal LDL-Cholesterol and Almost Die of a Heart Attack?"

Cardiovascular diseases (CVDs) are the leading cause of death globally (World Health Organization 2021). Lp(a), pronounced "Lp little a," is an LDL (low-density lipoprotein)-like, fatty, sticky lipoprotein particle with an additional protein apolipoprotein(a) [apo(a)] wrapped around it and found in blood serum. It is an inherited atherogenic lipoprotein and an independent risk factor for atherosclerotic cardiovascular disease, vascular thrombosis, stroke, and calcific aortic stenosis (Nordestgaard et al. 2010; Bennet et al. 2088; Erqou et al. 2009; Kampstrup et al. 2009; Boffa and Koschinsky 2016; Rogers and Aikawa 2015; Langsted et al. 2019). Lp(a) is one of the strongest genetically determined risk factors for cardiovascular disease (Kronenberg and Utermann 2013; CARDIoGRAMplusC4D Consortium and Deloukas 2013; Thanassoulis et al. 2013). Approximately 1 in 5 people have inherited high Lp(a), more than 1 billion people globally and 63 million in the United States who are unaware they have up to a 60% increased risk for coronary artery disease (Nordestgaard et al. 2010; Kamstrup et al. 2009). High Lp(a) is 80–90% genetically determined (Schmidt et al. 2016). Diet and exercise have little to no impact on high Lp(a) (Mackinnon et al. 1997). Most people fully express the LPA gene by the time they are 2 years old, reach adult levels by five, and for the most part maintain the same Lp(a) levels for a lifetime, although Lp(a) levels tend to increase with age in females after menopause (Wilson et al. 2019; Bittner 2002). Unfortunately, there are no visible symptoms, such as xanthomas, to indicate high Lp(a), and traditional cholesterol tests miss 8% of people who have a cardiovascular event whose only risk factor is high Lp(a) (Mortensen et al. 2015; Bittner 2015).

S. R. Tremulis (✉)
Redwood City, CA, USA

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_24

The traditional cardiovascular lipid screening does not include high Lp(a). However, a simple blood test performed **once** in a person's lifetime could be the first step in preventing up to 120,000 cardiovascular events every year (Wilson et al. 2019; Mortensen et al. 2015). Unfortunately, the first sign of cardiovascular disease often is a heart attack or a stroke. In 2003, I almost died of a heart attack despite having a healthy lifestyle, annual preventative health screenings, and no significant risk factors except my family history of cardiovascular disease. Bloodwork ordered after my heart event identified high Lp(a) as the only potentially significant contributing risk factor. I, therefore, propose expanding the current standard cardiovascular prevention lipid screening panel to include high Lp(a) testing for everyone to provide patients, families, and their healthcare providers with a more accurate prediction of their overall risk for premature cardiovascular disease and death (Wilson et al. 2019; Mortensen et al. 2015; Bittner 2015).

Reveal Lp(a): "You Must Have Had Some Really Bad Habits When You Were Younger"

Heart disease is the **leading cause of death** for men, women, and people of most racial and ethnic groups in the United States (Table 24.1) (Centers for Disease Control and Prevention 2018) and costs the United States about **$363 billion** each year from 2016 to 2017 (Virani et al. 2021). This includes the cost of healthcare services, medicines, and lost productivity due to death. There is no cure for cardiovascular disease; it is a chronic, systemic disease.

These daunting heart disease statistics have provoked fear in me for decades. My father died young of a heart attack; he had his first heart event at age 30, femoral bypass surgery at age 40, and a fatal heart attack at age 50. I was 22 years of age when he died. I never got over it; I just adjusted to it. Do you have a family history of cardiovascular disease? What are your personal inherited cardiometabolic risk factors for cardiovascular disease? I thought, heart attack, it was never going to

Table 24.1 2020 Top causes of death in the United States—Centers for Disease Control

2020 Top causes of death in the United States—Centers for Disease Control
Heart disease: 696,962
Cancer: 602,350
COVID-19: 350,831
Accidents: 200,955
Stroke (cerebrovascular diseases): 160,264
Alzheimer's disease: 134,242

The table was created from data in Mortality in the United States, 2020, data table for Figure 4 Murphy SL, Kochanek KD, Xu JQ, Arias E. Mortality in the United States, 2020. NCHS Data Brief, no 427. Hyattsville, MD: National Center for Health Statistics. 2021. DOI: https://doi.org/10.15620/cdc:112079. Copyright 2020 CDC/National Center for Health Statistics

happen to me. I was a young female, and I thought heart disease primarily impacted older men, like my father. I was in the medical device industry, knowledgeable about heart disease, and working on cutting-edge technology to help families faced with a cardiovascular disease diagnosis and helping to make a difference. I was proactive about my cardiovascular health. Because of my family history, I had a lifelong commitment to fitness and health, never missing my annual checkups. I thought I was doing everything right, and according to my Framingham and Reynold's risk score, I was!

Table 24.2 below is my Framingham Risk Score from 2003; it gave me a 1% chance of having a cardiovascular event.

Furthermore, Table 24.3 is my Reynolds Risk Score, an assessment that predicts cardiovascular disease, gave me a **1%** chance of having a cardiovascular event, and includes family history in their risk calculation algorithm.

However, based on the Bruneck Study, if you add Lp(a) to the Framingham and Reynolds Risk Score, I would have been reclassified with my high Lp(a) into a higher risk category like 20% of patients (Willeit et al. 2014).

In 2003, at 39 years of age, I went out for my usual 5-mile run, got one block down the road, and physically felt I could not go any further. I experienced fatigue and mild tingling in my chest upon exertion. Even still, like many people, I rationalized away my symptoms. I taught fitness classes as a hobby and had run a marathon the year before. I thought my tiredness was due to a potential thyroid issue, early

Table 24.2 Framingham Risk Score including Sandra Revill's data from 2003

Information about your risk score	
Age	39
Gender	Female
Total cholesterol	136 mg/dL (3.52 mm/L)
HDL cholesterol	30 mg/dL (0.77 mm/L)
Smoker	No
Systolic blood pressure	117 mm/Hg
On medication for HBP	No
Risk score[a]	*Less than* ***1%*** *The score means less than 1 in 100 people with this level of risk will have a heart attack in the next 10 years*
	[a]Your risk score was calculated using an equation. Other NCEP products, such as printed ATP III materials, use a point system to determine a risk score close to the equation score

The table was created from the Framingham Heart Study https://www.framinghamheartstudy.org/fhs-risk-functions/cardiovascular-disease-10-year-risk/using the 2003 personal medical data from Sandra Revill. Copyright for the Framingham Risk Score Calculator, D'agostino RB, Vasan RS, Pencina MJ, Wolf PA, Cobain M, Massaro JM, Kannel WB. General cardiovascular risk profile for use in primary care. Circulation. 2008 Feb 12;117:743–53. PMID:18212285

Table 24.3 Reynold's Risk Score including Sandra Revill's data from 2003

Information about your risk score	
Age	39[a]
Gender	Female
Total cholesterol	136 mg/dL (3.52 mm/L)
HDL cholesterol	30 mg/dL (0.77 mm/L)
Smoker	No
Systolic blood pressure	117 mm/Hg
High-sensitivity C-reactive protein (hsCRP)	0.16 mg/L
Did your mother or father have a heart attack before age 60?	Yes
Risk score	[a]Note the value you entered for age is outside the lower range. The result is based on age 45 As shown in the graph below, age 45, your chance of having a heart attack, stroke, or other heart disease event at some point in the next 10 years is **1%**

[a]The table was created from the Reynolds Risk Calculator, Calculating Heart and Stroke Risk for Men and Women http://www.reynoldsriskscore.org/Default.aspx using the 2003 personal medical data from Sandra Revill. Copyright for Reynolds Risk Calculator, Journal of the American Medical Association (Ridker PM, Buring JE, Rifai N, Cook NR. Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: The Reynolds Risk Score. JAMA 2007;297:611–619)

menopause, or the flu. It never occurred to me that I might have a heart problem. However, I knew something was wrong, so I scheduled an appointment with my family practice physician, who had a comprehensive overview of my family history of cardiovascular disease. The physician I saw wanted me to have a stress test in the emergency room, but they were too busy that day. Not thinking it was emergent, he suggested I was doing too much and recommended scheduling a treadmill test with the cardiology group. A couple of weeks later, I passed the treadmill test and got approval to teach my indoor cycling class that evening. Nevertheless, something was still wrong; I had to stop riding three times during class due to an overwhelming feeling of fatigue.

I still thought I had the flu! Later that week, I left on a business trip to Washington, DC, to attend a major medical device conference with all the top cardiologists in the world. I exercised in the hotel, as I usually did, but this time I felt terrible. I had the same fatigue and mild tingling in my chest as I ran on the treadmill, and when I slowed down, the tingling went away. I had swollen feet, so I started to take aspirin to try and reduce the swelling in my feet. I was aware of the risk of blood clots when flying long distances and that aspirin could help reduce that risk.

After I arrived home, compelled to seek a second opinion, I referred myself to an interventional cardiologist I knew in my professional role as a product marketing manager in the vascular business of a major medical device company. He listened to my history and informed me that he would be conservative and order a Nuclear

Stress Test because of my family history and symptoms. He felt I might have exercise-induced angina. I completed the Nuclear Stress Test, and I could not believe his diagnosis of potential cardiovascular disease. I had been physically fit my whole life and watched my diet because of losing my father at such an early age and diligent about my annual medical screenings, so what had I done wrong? He wanted me to take blood thinners overnight. He stated, "There are several abnormalities on your test, and I need you in hospital first thing tomorrow morning for a heart catheterization." I arrived at the hospital and was shocked when they asked me if I had a Will. I was a young, single female who owned property and had never dreamed I would need a Will at my age. I felt a sense of impending doom as I signed the consent papers at check-in to immediately convert me to bypass surgery should it be necessary if they could not stent the potential blockages in my heart arteries. I knew there might be limitations reaching the blockages with the current portfolio of stents because of my smaller female anatomy. I was facing this life crisis alone; my family was overseas without time to reach me. Petrified, they wheeled me down to the catheterization lab for the procedure.

Upon injection of dye into my coronary arteries, I heard a collective expression of surprise in the Cath Lab as it revealed a 95% occluded proximal left anterior descending coronary artery. This type of blockage is commonly referred to as the "widow maker" in the cardiology world. I remember asking, "Can you fix it?" The doctor said, "I think your father is sitting on your shoulder because I am not sure how you are still here." They inserted a drug-coated stent to open the blockage.

Interestingly, after my procedure, the Cath Lab nurses asked me, "Strong family history?" As I think about my journey with heart disease, they were a few of the people with empathy who instinctively, based on their experience, realized the inherited nature of my premature heart disease. During my follow-up visit with my cardiologist, I cried, and I asked him, "What did I do wrong?"

I felt this way because of the public misconception that a poor lifestyle is the only reason people get cardiovascular disease.

TEST Lp(a): "Why Test for It If You Cannot Treat It?"

The cardiologist said he wanted to understand the cause of my premature heart disease and ran more cardiometabolic bloodwork. That is the moment I discovered I had high Lp(a). He told me I did not do anything wrong, and I inherited this from my parents. I had an uncontrollable genetic risk factor for my premature heart disease, but surprisingly, it was a huge relief not to carry the shame that I could have prevented this event somehow. Finally, I could give my disorder a name. I was able to have a sense of control over an uncontrollable situation. He also assured me there would be significant advances in heart disease and not worry about my future.

Some would argue that testing for high Lp(a) is pointless in that "why test for it if you cannot treat it." Others would argue the benefit that testing for high Lp(a) uncovers a hidden genetic risk factor. I am one of the faces of high Lp(a) who had

an inaccurate prediction of my cardiovascular disease risk and feel that Lp(a) testing provides patients and their families a better risk prediction for premature cardiovascular disease and death. A personalized prescription for more aggressive primary or secondary prevention, including optimizing all cardiometabolic risk factors, can be initiated for an at-risk individual. Due to the thrombogenic nature of Lp(a), the doctor thought the aspirin I took when I felt my symptoms may have saved my life on the plane journey home by reducing the risk of a flow-limiting coronary thrombosis and a heart attack. In the Women's Health Study, carriers of the rare LPA gene variant (rs3798220) had a relative 56% risk reduction in ASCVD risk in carriers on aspirin therapy versus noncarriers (Chasman et al. 2009). More research should be conducted to improve the risk calculators and on aspirin use for primary and secondary prevention for patients with high Lp(a) (Mortensen et al. 2015; Zheng and Roddick 2019). I would also support risk-based versus trial-based calculators because many other factors decide enrollment criteria in randomized controlled clinical trials.

Over the next 10 years after my stent procedure, I would go through the grieving process for my former self as I recovered and returned to my new normal. I became an advocate for women's heart disease, but little information was shared about inherited cardiovascular disease. During these years, I had a child, and just as there is a 30-year deficit of data on women and heart disease, there was a total deficit of data to manage a woman through the reproductive years of her life with diagnosed heart disease and high Lp(a). Nevertheless, this was the beginning of my journey to learn about high Lp(a) and become educated and empowered and protect my own life. I lived with the trauma from my father's death and from my event, but it appeared nothing had advanced in the field of Lp(a) research in the 10 years since my heart event, and I wanted to know why. I later learned that the lack of implementation of a US and global standardized Lp(a) assay had hampered the progression of Lp(a) research, but now there are exciting new developments with a mass spectrometry-based approach for Lp(a) measurement. I would support the rapid adoption of a global standardized Lp(a) assay because time is measured in lives for patients.

A pivotal moment occurred when I went to see a leading lipid researcher at a major medical institution for a consultation. I wanted to know about the latest research on Lp(a). The appointment with the consultant took 2 h and cost $600. He concluded with a very clinical and dogmatic statement, "You have a malignant family history; there is no treatment for what you have. It is prohibitively expensive to research because each different ethnic group has a different normal level of Lp(a), and the child you risked your life having has a 50% chance of inheriting it!" I was motivated to make a difference and said to my husband, "I have nothing to lose except my life and everything to gain; I want us to make a difference. I do not want another family to suffer. I want to save lives by educating everyone about the health consequences of high Lp(a) and empower them to take action to reduce their risk and save lives." So, in 2013, I founded the Lipoprotein(a) Foundation.

Educate, Empower, and Save Lives: "I Wish All My Patients Were Like You and Engaged in Their Care"

I reviewed the published research focusing on evidence-based data and found that 20% (one in five people) have inherited high Lp(a), the most prevalent genetic risk factor for cardiovascular disease; more than one billion people worldwide are unaware they have at least a 60% increased risk for cardiovascular disease or death (Nordestgaard et al. 2010; Kampstrup et al. 2009; Emerging Risk Factors Collaboration et al. 2009; Patel et al. 2021). This lack of awareness was an unacceptable situation that could not continue. The first sign of the disease, for some people, is a heart attack or stroke. More than one billion families worldwide are unaware of their actual risk. I thought I was rare and an outlier, but, as I discovered, high Lp(a) is not a rare disorder (Nordestgaard et al. 2010).

When I mention these statistics, most people are shocked. Repeatedly, I hear heart disease is 80% preventable, but what about the 20% who have inherited an uncontrollable risk factor such as high Lp(a), familial hypercholesterolemia, hypertriglyceridemia, and homocysteinemia and their age or gender? During my research, it became increasingly clear we needed a dedicated charity to raise awareness and help educate families about their genetic risk from the fatty, sticky, Lp(a) particle in their blood on which diet and exercise have little to no impact. These families, including friends, neighbors, or loved ones, may not die from this inherited Lp(a) risk if we fund more awareness, advocacy, community support, and research programs. Very few people talk about inherited cardiometabolic disease, and even fewer people are diagnosed with it. I would support adding comprehensive cardiometabolic genetic testing to the risk calculators.

Our promise of value to our members was as follows:

> "Guided by evidenced-based data on Lp(a), we help educate and empower our members to save lives."

Our vision was as follows:

> "To live in a world where high Lp(a) is routinely diagnosed, treated, and family screened."

Our mission was as follows:

> "To reveal high Lp(a) as an inherited lipid risk for premature cardiovascular disease; educate and empower patients and save lives."

Our inaugural 5-year strategy in 2013 was as follows:

"To save lives by increasing awareness, advocating for routine testing, and a specific treatment for high Lp(a)."

What We Know: The Facts

- Fifty percent of hospital admissions for coronary artery disease have a normal LDL-C <100 mg/dL (Sachdeva et al. 2009).
- Lp(a) is currently the strongest, single genetic risk factor for coronary heart disease and aortic stenosis (Kronenberg and Utermann 2013).
- Increasing evidence reveals that high Lp(a) is a genetic, independent, and causal risk factor for coronary artery heart disease, atherosclerosis, thrombosis, stroke, and aortic stenosis (Nordestgaard et al. 2010).
- Approximately sixty-three million people in the United States are unaware of their risk from high Lp(a), one in five Americans and more than a one billion people globally (Nordestgaard et al. 2010).
- The Lp(a) blood test is not part of the regular lipid panel.
- Traditional lifestyle preventative measures including diet and exercise have little or no impact on Lp(a) levels (Mackinnon et al. 1997).
- High Lp(a) levels occur in all ethnic groups, but it is more common among African Americans, South Asians, and Hispanics (Paré et al. 2019).

What We Know: The Clinical Evidence

The increasing clinical evidence high Lp(a) is a causal risk factor for cardiovascular disease and calcific aortic stenosis.

- Epidemiological studies/meta-analyses (Emerging Risk Factors Collaboration et al. 2009)
- Mendelian randomized studies (Kampstrup et al. 2009)
- Genetic association studies (Clarke et al. 2009)
- Insights from UK Biobank (Patel et al. 2021)

Randomized controlled clinical trials (RCT)—patients with high Lp(a) levels are randomized to potential therapy. As of 2019, there are now at least three clinical trials underway for a specific therapy to lower Lp(a) (Viney et al. 2016).

Kare Berg discovered Lp(a) in human serum in 1963. After 60 years, there still is no FDA-approved therapy for lowering high Lp(a). With the launch of recent Lp(a) clinical trials, there is hope on the horizon for patients with high Lp(a).

The Lipoprotein(a) Foundation was a patient-founded and patient-focused organization that helped reveal the impact of high Lp(a). It was supported by a team of researchers, healthcare practitioners, and patient advocates who volunteered their knowledge and passion for helping others. We were honored to have Lp(a) key opinion leaders, both from the US and international arena, sharing their expertise and research insights with the Lipoprotein(a) Foundation. Our success was rooted in passion, empathy, innovation, and commitment. The foundation took pride in its innovative and grassroots approach to making real change for 20% of the global population

living with or at risk of cardiovascular disease or death due to high Lp(a). Based on feedback from our member community, I identified a set of strategic program areas for the foundation. Specific objectives were set within each program to help overcome the barriers to adoption for Lp(a) testing, which included executing an integrated marketing and communications plan within 5 years prioritizing grassroots efforts due to minimal funding and resources. The Lipoprotein(a) Foundation and its community made a measurable impact in these key strategic areas. Since 2013, the Lipoprotein(a) Foundation has delivered impactful programs driving awareness, advocacy, community support, and research to effect change and address unmet needs (Table 24.4).

Table 24.4 Overview of the foundation's key accomplishments from 2013 to 2020 (The Lipoprotein(a) Foundation 2020)

Then (2013)	Now (2020)
No ICD codes for Lp(a)	ICD-CM Codes E78.41 and Z83.430 approved—56% increase in individuals and 71% increase in families diagnosed[a]
No Lp(a) contact registry	8000+ enrolled; helped enroll three phase 1 and one phase 2 clinical trials. Published market research study on participating in clinical trials during COVID-19 (Swerdlow et al. 2021)
No Lp(a) awareness	500+ million impressions from PR activities, 500+ online headline postings, top Google ranking, featured in *New York Times*, *USA Today*, *Fox News*, American Airlines, Martha Stewart Living, plus others. Community outreach focused on high-priority gender, ethnic, and disease state groups
No Lp(a) support community	Growing community online and in person—social media, patient forum program, community events, and support phone line
No Lp(a)-focused website	140,000+ visitors from 166 countries to the website each year offering Lp(a) expert physician location services
No professional guidelines	Lp(a) in ACC/AHA, NLA, ESC/EAS, and cholesterol guidelines as a risk factor (Virani et al. 2022)
No group advocating for Lp(a)	Seven years of Lp(a) advocacy with NIH, CDC, and others
Little attention and funding for Lp(a)	NIH strategic research proposal (Tsimikas et al. 2018)—$400K grant awarded to Columbia University Medical Center
No gathering of experts	Thirty top Lp(a) experts on SAB and CAB advisory board, including representation from all our prioritized groups
No treatment options	Five potential innovative treatments in development, three in clinical trials; helped enroll three phase 1 (Akcea/Ionis, Amgen, Silence Therapeutics) and one phase 2 clinical trial (Akcea/Ionis/Novartis)
No directory of Lp(a) specialists	600+ physicians registered with the foundation
No standardized Lp(a) blood test	NHLBI/CDC working group conducted on global standardization of Lp(a) assay in humans (Lijuan et al. 2019)

The table was created from data from the 2019 Impact Report for the Lipoprotein(a) Foundation L00012US 6/20. Copyright 2020 Lipoprotein(a) Foundation EIN: 46-3024812 a nonprofit, 501(c)3 patient advocacy organization

[a]Data provided by Vladimir Polony from the Green Button team to the Lipoprotein(a) Foundation, led by Nigam Shah at the Stanford Center for Biomedical Informatics Research from a representative sample database

Patients and their families need an accurate prediction of their risk for premature cardiovascular disease to prevent the first symptom from being death. A 2016 study by Mortensen et al. looked at statin eligibility and 5-year cardiovascular disease outcomes in 37,892 individuals (57% women) aged 40–75 years of age in the Copenhagen General Population Study (Mortensen et al. 2015). The study limitations include that it only looked at Caucasian subjects and was limited to a 5-year follow-up. It would have been more informative to include higher-risk Hispanic, Black, and South Asian populations with high Lp(a). The study used the 2013 American College of Cardiology/American Heart Association (ACC/AHA) risk prediction tool. In the results of their study, as noted by Dr. Vera Bittner (the University of Alabama at Birmingham) in an accompanying editorial, "The study suggests that Lp(a) levels might help identify the 8% of individuals who had an event despite being ineligible for statins." She noted, "Comprehensive risk factor control is associated with improved prognosis, and our challenge is to develop care models that will allow us to achieve such control." Another perspective accompanying the article notes, "Future research should be directed toward developing more accurate risk prediction tools." In the editorial accompanying this study, Dr. Valentin Fuster, JACC editor-in-chief (Icahn School of Medicine at Mount Sinai, New York), suggested, "Let's begin to pay attention to high Lp(a) because it may explain cardiovascular events in patients who otherwise do not have a significant risk factor profile." (Bennet et al. 2088) There are 1.5 million people in the United States who have a cardiovascular event every year; 8% of that number is 120,000 people (Erqou et al. 2009).

The public appears to have little empathy for cardiovascular disease because it is often perceived as self-inflicted. But would not it be good to reduce the emotional and financial impact on US society of an estimated 120,000 people with only isolated high Lp(a) having a cardiovascular event every year and many more globally? Individuals can be diagnosed with a simple inexpensive Lp(a) blood test, but you can also identify a family potentially at risk for generations to come. It is a simple, blood test once in a person's life and annual bloodwork is not required. We encourage healthcare practitioners to pursue continued education about this inherited lipid risk. In medical practices, one in five individuals and their families already have high Lp(a) and face at least a 60% increased risk of a cardiovascular event (Nordestgaard et al. 2010; Kamstrup et al. 2009). Educating and empowering patients about high Lp(a) does save lives. In 2019, the Lipoprotein(a) Foundation was named a Top-Ranked Nonprofit by the leading platform for community-sourced stories about nonprofits. The foundation received this award for successfully achieving the objectives of our 5-year strategic plan and because of community feedback on our programs. A patient advocate, stated, "The Lipoprotein(a) Foundation has helped save my life! It was the beginning of my journey, guiding me through what I needed to test to identify what ended up being significant heart disease." This testimonial was just one of the many mission moments that occurred as we fulfilled our objectives for the foundation.

Conclusion

Awareness

So, how can I have had normal LDL-cholesterol and almost died of a heart attack despite having a healthy lifestyle, annual preventative health screening, and no significant risk factors except my family history of cardiovascular disease? It is my opinion that, unfortunately, there is low public awareness for personal inherited cardiovascular disease risk (Sanderson et al. 2011). The data to generate that awareness for high Lp(a) has been inconsistent and largely missing due to the lack of a standardized Lp(a) assay for research purposes, drug target development, and level 1 data from clinical trials for a therapy to improve outcomes for patients. Level 1 data is the trigger to include a risk target into the global cholesterol guidelines if they are trial-based versus risk-based, and those guidelines are periodically updated (Marcovina et al. 2003). Often, the public perception is that cardiovascular disease is entirely self-inflicted, and the stigma attached to it is similar to AIDS and lung cancer, which reduces the funding and empathy that often drives awareness of a disease state (Benson 2021). This is also the case for women's heart disease due to a 30-year deficit of women and heart disease data (Garcia et al. 2016). I was aware of my family history of heart disease but not my risk as a young woman with a strong family history. The global focus and funding imperative for COVID-19 vaccines show how an enormous-focused response might finally eliminate the insurmountable global burden of cardiovascular disease. All stakeholders involved in the cardiometabolic disease industry should focus on driving awareness for personalized, inherited cardiometabolic disease.

Women's Heart Disease Data

In addition, we must do better for women in healthcare and recognize the unique and important differences between men and women; women's more subtle symptoms may be ignored or treated less aggressively than male patients (Garcia et al. 2016). I was one of those women with subtle symptoms treated less aggressively. Many biases can impair diagnostic accuracy by humans. Availability bias, a cognitive bias, can lead to diagnosis errors (Yagoda 2018).

Access to Latest Top-Quality Evidenced-Based Data

Without having the latest medical research data available on-demand to healthcare providers at the point of care within their institution, a physician cannot be informed about the latest evidence-based data to aid in care (Lenaerts et al. 2021). With the

advent of artificial intelligence (AI), there is promise for AI tools such as the Human Diagnosis Project, also known as Human Dx, aiding in diagnosis if the human biases do not become embedded in the AI tools (Human Diagnosis Project 2022).

Clinical Centers of Excellence for High Lp(a)

It would benefit patients and their families to establish focused clinical centers of excellence providing equal access to specialized treatment and care for inherited cardiometabolic disease with a priority given to underserved minority groups at increased risk from high Lp(a). Developing a standard of care to direct families with inherited cardiometabolic disorders to these clinical centers of excellence would simplify access to state-of-the-art research and care (Elrod and Fortenberry Jr. 2017).

Lack of Standard of Care for High Lp(a)

There was no standard of care to manage me through my life or reproductive years with high Lp(a). Including reproductive risk factors as part of cardiovascular risk assessment in clinical guidelines would help identify women at risk. Identifying reproductive risk factors such as amenorrhea, polycystic ovary syndrome, thyroid disorders, pregnancy loss, and pregnancy complications at an early stage in a women's life might provide a more accurate prediction of risk and facilitate the initiation of strategies to modify potential risks. Including gynecologists on the care team for women attending a clinical center of excellence for inherited cardiovascular disease would provide a more comprehensive view of a women's potential lifelong risk (Garcia et al. 2016).

Rapid Deployment of a Globally Standardized Assay for High Lp(a)

We have known about high Lp(a) for 60 years and still do not have a globally standardized Lp(a) assay, which has hampered research and the progression of the body of scientific evidence in this area (Marcovina et al. 2003). The bloodwork identifying my only inherited, hidden, significant contributing risk factor, high Lp(a), was performed after my life-threatening event. Including a globally standardized test for high Lp(a) in the standard preventative lipid screening and risk calculators would provide a more accurate prediction of risk for patients and their families, initiating more aggressive primary and secondary prevention, which otherwise may not have been identified (Mortensen et al. 2015).

Improved Risk Calculators

There is no path of vigilance for inherited high Lp(a) as there is for other diseases from birth onward. The risk calculators that guide the standard of care for cardiovascular disease are designed for population medical care and not personalized medicine. They do not include inherited cardiometabolic risk markers such as high Lp(a) or factor in premature cardiovascular disease at an age younger than 45 (Semaev and Shakhtshneider 2020). I was one of those young people with high Lp(a) missed by the risk calculators.

Precision Medicine

A prescription for cardiovascular disease prevention specifically tailored for the individual and their genes is needed. The healthcare industry should remove financial penalties for patients diagnosed with an inherited risk. Instead, reward patients for seeking to understand their risk for inherited cardiometabolic disease and take action to optimize all their cardiometabolic risk factors. Patients pay for the testing and care to build their natural history data in institutional databases. Allowing patients to own and monetize their natural history data and be informed of the research developments derived from that data would help expedite research participation. It would make the patient a true stakeholder in the research development process.

Aspirin for Event Prevention for Patients with High Lp(a)

Personalized preventative medical care is costly to adopt for the general population. Researching the benefit of aspirin use for high Lp(a), including aspirin resistance and other forms of blood clotting disorders, could provide a cost-effective preventative solution for patients with high Lp(a) (Greving et al. 2008). It will not prevent cardiovascular disease due to high Lp(a) but might save lives. By chance, I took aspirin during my heart event due to public awareness information about blood clots when flying.

National Database for Cardiovascular Disease

Developing a comprehensive national database of premature cardiovascular events, as there is for cancer, accessible to all researchers would also help expedite research, improve standards of care, and draw public attention to the emotional and financial

burden of premature cardiovascular disease in the United Sates and help prioritize funding (Bilimoria et al. 2008). The healthcare industry could invest savings generated by preventing cardiovascular events and heart damage for 20% of the global population with high Lp(a) into the cost of precision, preventative cardiometabolic care. Improving the survival rate of a heart event is good but often moves the costs along to managing the chronic condition of heart failure after the heart is damaged (Heidenreich et al. 2013).

Measurable Impact But Still More Work to Be Done

The Lipoprotein(a) Foundation provided impactful programs to effect change to prevent families from suffering the same fate as my family. There is still much work to be done to improve the healthcare process for patients with high Lp(a) and our knowledge of the overall health consequences of high Lp(a). However, the foundation made a measurable impact on awareness, advocacy for the rapid deployment of a standardized assay, improvements in the risk calculators, screening, and diagnosis for inherited high Lp(a), expediting the development of therapies and educating and empowering the healthcare community and the public to help save lives with very limited funding and resources (The Lipoprotein(a) Foundation 2020).

Unfortunately, on July 31, 2020, the Lipoprotein(a) Foundation dissolved after a 90% reduction in donations due to COVID-19 and increasing costs. I was honored to represent the more than one billion people globally living with or at risk of cardiovascular disease due to high Lp(a). My educational journey with high Lp(a) continues as more research identifies new insights into high Lp(a) and the origins of my inherited immuno-cardiometabolic risk. I am an educated and empowered individual who actively participates in their care. I dedicate this chapter to my family and all those families with a history of inherited cardiometabolic disease and Dr. Tomoaki Hinohara for saving my life.

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Chapter 25
Unresolved Questions

Gerhard M. Kostner and Karam Kostner

It is now almost exactly 60 years since Kare Berg first described an extra pre-β band found in lipid electrophoresis that later was named sinking pre-β and finally Lp(a). There was a continuous up and down in Lp(a) research that was mainly driven by actual research findings related to Lp(a) function, metabolism, correlation to cardiac risk, and epidemiology. The following are four key findings that caused a major boost in the interest for Lp(a) that led to a flurry in publications:

1. Cloning of *LPA* by McLean and Lawn demonstrating homology of the apo(a) protein and the *LPA* gene with plasminogen (McLean et al. 1987; Utermann 2001).
2. The unique size polymorphism caused by variations in the number of K-IV2 repeats that paved the way for consecutive genetic epidemiological studies by the group of Utermann [reviewed in (Utermann 2001; Kamstrup et al. 2009)].
3. The demonstration of the causal relationship of elevated Lp(a) levels with atherosclerosis and coronary heart diseases by Mendelian randomization in the Copenhagen Heart Study (Kamstrup et al. 2009; Graham et al. 2016).
4. The development of a very efficient therapy for elevated-Lp(a) with antisense oligonucleotide (ASO) therapy by the group of Tsimikas (Graham et al. 2016).

All these exciting milestones in Lp(a) research cannot change the fact that our knowledge in all areas of Lp(a) research is still fragmented. This is due to a lack of knowledge in the following areas:

1. Function
2. Metabolism

G. M. Kostner (✉)
Institute of Molecular Biology and Biochemistry, Medical University of Graz, Graz, Austria
e-mail: gerhard.kostner@medunigraz.at

K. Kostner
Department of Cardiology, Mater Hospital and University of Queensland, Brisbane, Australia

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_25

3. Pathophysiology
4. Lp(a) measurement in clinical laboratories
5. Significance in diseases other than related to atherosclerosis
6. Therapy

Many of these points have been already discussed in the previous chapters and will therefore only be summarized here:

Is There a Function of Lp(a) in Longevity and Suppression of Malignant Growth?

Nature rarely designs complex structures such as apo(a) without any physiological function in mind. This may not necessarily be true for the whole human population but may be only for some ethnicity. Thus, it has been speculated that individuals exposed to dangerous parasites or bacterial and viral infections may have an advantage if they have high Lp(a) plasma concentrations. The actual mechanism behind this is far from being clear, yet it may explain why populations originating from African countries where such diseases prevail have significantly higher Lp(a) levels than Europeans and Asians (Schmidt et al. 2006; Sandholzer et al. 1992).

Another function of Lp(a) might relate to aging and longevity. In fact, lipids and lipoproteins have been implicated in life span regulation (Joshi et al. 2017), and in the list of genes suggested in previous research to code for such factors, *LPA*, *APOE*, and *APOAI* are found. We addressed this question in early investigations in view of age-related diseases and hypothesized that assuming that Lp(a) might be a significant risk factor for atherosclerosis and MI, individuals with high Lp(a) should die earlier than individuals with low Lp(a). Thus, we first measured Lp(a) in a family kindred within three generations and anticipated that Lp(a) values in the older generation might be lower than in the younger one (Pagnan et al. 1982). In fact, the opposite turned out to be the case. In another study, we measured Lp(a) in octononagenarians (Zuliani et al. 1995), and although we could not confirm a correlation of Lp(a) with age, to our surprise, the plasma Lp(a) concentration of "very old" individuals and more importantly the apo(a) isoform distribution did not differ significantly from that of young individuals (Zuliani et al. 1995). Comparable studies have been also published from other investigators (Wood and Schumacher 1995).

The question obviously arises about the physiological relevance of these observations—or in other words—does this relate to a physiological function of Lp(a). We studied this possibility by asking whether apo(a) might be involved in angiogenesis (Schulter et al. 2001). Angiogenesis has been found to be important not only for tumor growth but also for cancer metastasis. O'Reilly et al. (1994) were first to demonstrate that angiostatin, a proteolytic cleavage product of plasminogen secreted into urine, has very high angiostatic properties. Since proteolytic fragments from apo(a) are found in urine as well, we purified these fragments and tested their angiostatic properties in vitro in a tube forming assay: indeed apo(a) from urine that

consist mainly of N-terminal fragments exhibited a significant reduction of tubes in the Matrigel assay. In another study, transgenic apo(a) mice and control mice were injected with 10^7 Ehrlich ascite cells that form solid tumors within 4 months. The number and size of solid tumors in tg-*APOA* mice were significantly lower as compared to control mice. Whether or not these findings are applicable to humans in vivo remains to be demonstrated.

Another physiological function of Lp(a) might relate to its high binding capacity of secretory phospholipases (sPL-A2), PAF acetyl hydrolase (PAF-AH), and oxidized phospholipids (OxPhos). We were among the first to demonstrate that Lp(a) carries a three times higher activity of phospholipase-A2 as compared to LDL (Gorges et al. 1995). In addition, a manifold higher PAF-AH activity compared to LDL has been demonstrated in Lp(a) (Blencowe et al. 1995). This is probably one reason why Lp(a) is less susceptible to oxidation than LDL (Sattler et al. 1991). The transport of OxPhos by Lp(a) was suggested to be the major culprit for its pathomechanism in atherogenesis: OxPhos Lp(a) complexes that enter the arterial intima—particularly when other risk factors such as high LDL are abundant—trigger inflammatory processes, recruitment of lymphocytes and cytokines, foam cell formation, and all the well-described features of atherosclerosis and heart diseases. On the other hand, nature seldom produces pathogenic substances for fun, and we hypothesize that the absence of atherogenic bystanders Lp(a) might be beneficial and counteracts the development of cancer. This might relate to the mentioned interference with angiogenesis on one hand and to the Lp(a)-OxPhos-phospholipase pathway on the other hand. Phospholipase-A2—and in particular PAF-AH—cleave and then inactivate free radicals and hydroperoxides found on short-chain fatty acids of phospholipids. The latter substances have been found to trigger cancerogenesis by creating an inflammatory milieu, chemokine attraction, signaling, and cell growth (Hermann et al. 2014). Alternatively, PL-A2 receptor-1 that is found on the surface of numerous cancer cells has been suggested to possess tumor suppressor activity by interaction with certain phospholipases (Sukocheva et al. 2019).

Taken together, there is a great deal of speculations about the physiological function and possible beneficial roles of Lp(a), and this needs to be addressed in future research.

Metabolism

Biosynthesis and assembly: The chapter authored by Dan Rader and John Miller in this book gives an excellent overview on the current concepts of Lp(a) metabolism. As these authors point out, our research group was first to demonstrate that the Lp(a) metabolism is distinct from that of LDL: other than for LDL, VLDL is not a precursor of Lp(a) (Krempler et al. 1979). We also published that other than for LDL, plasma Lp(a) concentrations are governed by the rate of biosynthesis—or in other words, individuals with high Lp(a) concentrations show a high rate of apo(a) expression (Krempler et al. 1980). The expression of apo(a) is driven by

transcription factors and nuclear receptors. In silico search in the *APOA* promoter revealed more than 70 binding regions for known transcription factors; two of such response elements, ETS −1630 to −1615 and DR-1 −826 to −814, turned out to be of particular importance as they are strongly turned off by FXR signaling (Chennamsetty et al. 2011). We also identified several cAMP response elements that were responsible for the Lp(a) lowering effect of nicotinic acid (Chennamsetty et al. 2012). But how about the role of all the other response elements that we identified in the apo(a) promoter? This is an ample research field that deserves much further attention.

Following *APOA* transcription, translation, and glycosylation, the mature apo(a) protein assembles with LDL to form Lp(a). The individual steps in assembly have been addressed in numerous studies in the past, yet there is currently no general agreement on the site where this might occur. Whereas some data favor an intrahepatic assembly, other data point toward an assembly on the surface of liver cells, and even others suggest an assembly in circulating blood. Undoubtedly there are further studies needed to clarify the location of the assembly of Lp(a).

Another fully open field is the role of the *APOA* expression in the brain and testes (McLean et al. 1987): does this have any physiological relevance? Nobody has ever studied this rather interesting phenomenon in detail.

Catabolism: In our early experiments in man, we found that FH patients lacking LDL receptors catabolize Lp(a) to the same extent than healthy controls. The catabolic rate in both, however, was markedly slower as compared to LDL (Krempler et al. 1980). This led us to conclude that LDL receptor-mediated catabolism plays little role in Lp(a) removal from circulation. Since then, a wealth of publications appeared that found Lp(a) binding to almost any specific lipoprotein receptor including the apoE receptor, the VLDL receptor, the remnant receptor, LRP receptor, the asialoglycoprotein receptor, the plasminogen receptor, several scavenger receptors, and possibly others. Fact is that in all animal studies even in hedge hogs, approximately 50% of intravenously injected Lp(a) is taken up by the liver (Kostner et al. 1997). In addition to the liver, also the kidney plays an important role in Lp(a) metabolism (see chapter of H. Dieplinger in this book). Apo(a) is fragmented in the blood by Ca^{2+}-dependent proteases, and even large fragments are secreted into urine (Frank et al. 2001). The significance of this pathway has never been clarified so far.

Pathophysiology: What Are the Most Important Determinants of Lp(a) Pathogenicity?

We know more about the pathophysiology of Lp(a) than about its physiology. Lp(a) consists of an LDL particle with all its proatherogenic properties. In addition, Lp(a) gets into atherosclerotic plaques by interacting with proteoglycans, which causes foam cell formation and inflammation; Lp(a) also carries OxPhos that trigger inflammation. Due to the homology of apo(a) with plasminogen, Lp(a) has also

been connected to fibrinolysis and thrombosis [reviewed in (Boffa 2022)]: on one hand, it was suggested that Lp(a) interferes with the conversion of plasminogen to plasmin by plasminogen activator and urokinase, and on the other hand, it interferes with the action of PAI on the endothelial surface. Further studies revealed that Lp(a) is incorporated into fibrin clots and aggravates the action of plasmin in fibrinolysis. Considering the complexity of hemostasis and fibrinolysis that involve numerous significant components working in a concerted action to prevent bleeding on one hand and uncontrolled blood clotting on the other, it seems questionable that Lp(a) plays a significant role in these pathways—and if it does—this certainly needs further clarification.

Lp(a) Analysis in Clinical Laboratories

The early laboratory methods for measuring Lp(a) were based on immunochemistry. Almost all immunochemical methods including radial immune-diffusion (Ouchterlony test), rocket electrophoresis, radioimmunoassay (RIA), ELISA (enzyme-linked immunosorbent assay), DELFIA (dissociation-enhanced lanthanide fluorescence immunoassay), and more methods have been applied. Today, high-throughput methods for Lp(a) quantitation are based on immune-turbidimetry or immune-nephelometry. Unfortunately, commercial methods are far from being harmonized and subject to drastic improvements. There is currently no validated reference material commercially available, and reference methods for typing Lp(a) standards are still under development. All the problems with Lp(a) quantitation in the clinical laboratory are impressively documented in the chapters from S. Marcovina and C. Cobbaert and D. Sullivan in this book. Another article that highlights this thematic was recently published by F. Kronenberg (Kronenberg 2022). Kronenberg looks at this topic from practical point of view and stresses the point that due to the great genetic heterogeneity of *LPA*, it will be hardly possible to have a validated routine method for high-throughput Lp(a) measurement at reasonable costs available that fulfills all standard requirements of ISO 17511:2020. Former studies where commercial assays for Lp(a) were evaluated revealed a significant number of outliers that at present time cannot be explained (Scharnagl et al. 2019). C. Cobbaert established an IFCC (International Federation of Clinical Chemistry)-sponsored working group with experts in the field of mass spectrometry and laboratory medicine with the goal to develop a reference method based on LC-MS (liquid chromatography-mass spectrometry) http://www.ifcc.org/ifcc-scientific-division/sd-working-groups/wg-apo-ms/. In addition, this group works on the preparation of a harmonized SI-traceable reference material that shall be used by industry to standardize their commercial assays. Although the IFCC working group has been operational for more than 5 years, it may take another 2–3 years to come up with a practicable reference method and a reference material.

Major hurdles in this study are the following

1. SI units in clinical chemistry need to be expressed in molar units, and this is hard to achieve for Lp(a) because of the large size heterogeneity ranging from 300 to 800 kD for apo(a). Also, apo(a) contains a variable number of identical K-IV2 repeats and in addition other homologous K-IV's cross reacting with polyclonal antibodies that are normally used for nephelometric Lp(a) assays. In order to overcome the bias created by these properties of apo(a), commercial assays use an algorithm for correcting the measured values—yet this is just an approximation and not correct for a large number of samples.
2. Even using a standardized reference method such as ELISA with monoclonal antibodies or a validated LC-MS method, there are numerous outliers observed in bias plots by comparing two or more methods. The reason behind is unknown and needs further research.
3. Not all apo(a) is complexed with LDL, and there is an appreciable amount of free apo(a) and apo(a) fragments found in plasma that might vary in concentration particularly in kidney disease and sepsis. These fractions are differentially measured in various assays but need to be considered for different assays.
4. A further question that needs to be answered beyond any doubt is the atherogenicity of large versus small apo(a) isoforms. There are quite a few papers published on this issue, but they are partly controversial mainly due to problems mentioned in (2) and (3). It is also not clear whether polymorphic or mutant forms of apo(a) are to the same extent atherogenic than "wild-type" apo(a).
5. What is the significance of the variation in lipid composition of Lp(a) from individual donors? As pointed out in the chapter of G. Kostner in this book, it turned out that Lp(a) in reality is far from being homogenous in its lipid part. This is corroborated by data from novel Lp(a) cholesterol assay stressed by C. Yeang (Yeang et al. 2021) who showed that Lp(a) cholesterol content in percent relative to the Lp(a) mass varies from 5.8 to 57.3% in his study group (Yeang et al. 2021). An interesting question would be whether the atherogenicity of Lp(a) relates to its cholesterol content or lipid composition.
6. Is there an easy way to quantify LDL-C without Lp(a)-C? So far, corrections for Lp(a)-C have mostly done—if at all—by subtracting 30% of the Lp(a) mass from LDL-C—yet considering the results from C. Yeang (Yeang et al. 2021), this gives quite striking erroneous results.

Summing up the open questions related to laboratory methods, our knowledge in this field is limited and deserves intensive future research.

Does Lp(a) Lowering Reduce Hard CV Endpoints?

Apart from LDL apheresis therapy, it is currently not clear whether lowering of Lp(a) reduces hard cardiovascular endpoints. Several phase 2 and 3 trials with antisense and siRNA-targeted therapies are exploring this currently. Most lipidologists

and clinicians recommend to lower LDL cholesterol more aggressively to levels below 100 mg/dL in case of elevated Lp(a) levels, even though the hard evidence for this is also lacking.

Pelacarsen is an antisense oligonucleotide that targets apo(a) mRNA. It has shown Lp(a) reductions of more than 80% and is being tested in a large phase 3 trial called HORIZON (Viney et al. 2016).

Olpasiran is an example of an siRNA targeting apo(a) mRNA that has shown to also reduce Lp(a) by more than 80% and is also being tested in a phase 2 study, with a phase 3 study planned for later this year (Koren et al. 2020).

These trials will help answer the question whether Lp(a) reduction leads to CV endpoint reduction.

How Much Should Lp(a) Be Lowered?

Mendelian randomization studies suggest that to achieve significant CV risk reduction, similar to what has been seen with LDL reduction of 1 mmol/L, Lp(a) would have to be reduced by 250 nmol/L. However, these studies were population based and included many patients with low Lp(a) levels, which would usually not be considered for Lp(a) lowering. In addition, if Lp(a) is more atherogenic than LDL, smaller reductions in Lp(a) may prove more important clinically. Data from recent PCSK9 inhibitor trials indicate that smaller reduction in Lp(a) may have a significant effect on CV endpoints.

In the FOURIER (Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk) trial, reduction in risk of MACE (major adverse cardiac event) with evolocumab was associated with baseline and change in Lp(a) levels (Gencer et al. 2021).

In the ODYSSEY OUTCOMES (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab) trial, reduction in risk of total cardiovascular events with alirocumab was also associated with baseline and change in Lp(a) levels (Bittner et al. 2020).

Reduction in risk of major adverse limb events (MALE) with alirocumab was also associated with baseline and change in Lp(a) levels.

In our opinion, Lp(a) should be lowered as low as possible in high-risk individuals.

Which Is the Most Effective Therapy to Lower Lp(a)?

LDL apheresis is effective and leads to significant Lp(a) reductions as well as HR reductions in observational studies but has not been tested in CV outcome trials. Apheresis is FDA approved and can be considered in patients with elevated Lp(a) at very high risk of ASCVD (atherosclerotic cardiovascular disease), but it is expensive, inconvenient, and not widely available (Moriarty et al. 2019). Emerging

antisense and RNA technologies are more specific and show much larger Lp(a) reductions. Clinical outcome trials are currently underway (HORIZON, NCT04023552; OCEAN(a)-DOSE; NCT04270760).

Monoclonal PCSK9 antibodies, as well as RNA-based inhibitors of PCSK9, which lower LDL-C, can also reduce Lp(a) by up to 35%. While they are not reimbursed through Medicare for Lp(a) treatment, elevated Lp(a) is often treated coincidentally in patients with FH or progressive ASCVD whose LDL-C remains elevated despite maximal statin and ezetimibe therapy. Sub-analysis of the major outcomes trials for PCSK9 inhibitors has shown greater relative and absolute risk reduction in patients with elevated Lp(a). In Cardiovascular Outcomes Research With PCSK9 Inhibition in Subjects With Elevated Risk (FOURIER) trial, reduction in risk of major acute coronary events (MACE) with evolocumab was associated with baseline and change in Lp(a) levels (O'Donoghue et al. 2019). In the Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment With Alirocumab (ODYSSEY OUTCOMES) trial, reduction in risk of total cardiovascular events with alirocumab was also associated with baseline and change in Lp(a) levels (Szarek et al. 2020). Reduction in risk of major adverse limb events (MALE) with alirocumab was also associated with baseline and change in Lp(a) levels (Schwartz et al. 2020). These trials support the conclusion that elevated Lp(a) is a major driver of residual risk.

The Effect of Statins on Plasma Lp(a) Levels

Statins do have a variable effect on plasma Lp(a). Although most statins are able to lower Lp(a) to some extent, there are numerous patients who do not respond to statins at all or even show an increase of Lp(a) on statin therapy (Kostner et al. 1989). The mechanisms responsible have never been defined. The important fact to remember is that statins are beneficial in patients with elevated Lp(a) by removing LDL, which reduces some of the CV risks associated with elevated Lp(a).

Who Should We Screen for Lp(a) and How?

Knowledge of Lp(a) could be particularly valuable in reclassification of patients at intermediate risk of ASCVD, as assessed by established risk algorithms. Most societies recommend that Lp(a) should be measured in individuals with a personal or family history of premature ASCVD (or aortic valve stenosis) and familial hypercholesterolemia (FH) or in those with recurrent coronary events despite optimal LDL cholesterol on diet and statins, with or without ezetimibe. Information on Lp(a) may guide more aggressive treatment of conventional risk factors or the need to assess subclinical atherosclerosis with cardiac CT scanning (Kostner et al. 2018).

We recognize the importance of elevated Lp(a) as a cardiovascular risk enhancer, particularly in light of the significant residual risk that remains despite reduced LDL. The precise value of cascade testing first-degree relatives of an index case with elevated Lp(a) has not been demonstrated. However, it could help define and consolidate the family history of ASCVD and improve adherence to existing therapies in secondary prevention, as well as to healthy lifestyle and behavior in primary prevention in family members. Elevated Lp(a) with a coexistent polygenic hypercholesterolemia or familial combined hyperlipidemia may mimic FH and should always be considered in patients who return a negative genetic test for FH.

There is a great deal of information available on Lp(a) physiology and pathophysiology that is published in more details, that is, >9000 10,000 scientific publications. This should not mislead that there are still numerous open questions and gaps in our knowledge, and we should capitalize the current hype in Lp(a) to address this topic more rigorously.

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Correction to: The Kidney Is the Heart of the Organs: Its Role in Lp(a) Physiology and Pathophysiology

Hans Dieplinger

Correction to:
Chapter 12 in: K. Kostner et al. (eds.),
***Lipoprotein(a), Contemporary Cardiology*,**
https://doi.org/10.1007/978-3-031-24575-6_12

The author requested that the DOI entries in the 'References' section be removed, but the book was inadvertently published with them included. The error has been corrected.

The updated original version of the chapter can be found at
https://doi.org/10.1007/978-3-031-24575-6_12

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K. Kostner et al. (eds.), *Lipoprotein(a)*, Contemporary Cardiology,
https://doi.org/10.1007/978-3-031-24575-6_26

Index

A

Acute coronary syndrome (ACS), 150–151, 276
Acute lymphoblastic lymphoma, 140
Ag system, 1, 40
Alirocumab, 351
All Lab Total Mean (ALTM), 335
American College of Cardiology (ACC), 278
American College of Cardiology/American Heart Association (ACC/AHA) risk prediction tool, 418
American Heart Association (AHA), 278
Angiogenesis, 141, 142, 147
Anticoagulation, 193, 199, 201
Anti-fibrinolytic mechanisms, 144
Antiplatelet agents, 199
Antisense oligonucleotide (ASO), 85, 86, 367
  antisense oligonucleotide pharmaceutical activity, 366
  development of, 363, 365
  generations of, 362, 363
  with IONIS-APO(a)LRx/AKCEA-APO(a) LRx,/TQJ230/Pelacarsen, 370
  ISIS-APO(a)Rx/IONIS-APO(a)Rx, 367
  Lp(a) HORIZON trial, 373, 374
  pre-clinical proof of, 360
Aortic leaflet valve interstitium, 328
Aortic stenosis (AS), 163
Apheresis, 352
Apo B100-containing particles, 283
Apo(a), 40, 42–53
  carbohydrate moiety, 47
  gene variants, 49
  isoforms, 57
  mutations, 51
  protein structures, 45–47
  structural features, 47
APOA transcription, 428
ApoE*3-Leiden CETP mouse model, 400
Apolipoprotein(a) (apo(a)), 8–15, 17–21, 23–25
Apolipoprotein B (ApoB), 382
  antisense oligonucleotides, 102
  lowering, 199–201
  synthesis/secretion, 85
Arterial vascular disease, 22
Artificial intelligence (AI), 420
ASCVD, *see* Atherosclerotic cardiovascular disease (ASCVD)
Asialoglycoprotein surface receptor (ASGPR), 130, 215, 363, 428
Aspirin, 414
Associations of Statutory Health Insurance Physicians, 379
Atherosclerosis, 262
  aortic stenosis, 163
  ASCVD, 160
  genetic studies, 165
  inflammation, 162
  mortality, 164
  myocardial infarctions, 163
  PAD, 164
  stroke, 163
  treatment, 166
Atherosclerotic cardiovascular disease (ASCVD), 160, 162, 173, 180, 191, 192, 199–201, 275, 409
Atherosclerotic profile, 161
Atherothrombosis, 191, 195, 199–201

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https://doi.org/10.1007/978-3-031-24575-6

Atherothrombosis Intervention in Metabolic syndrome with low HDL/high triglycerides: Impact on Global Health outcomes (AIM-HIGH) study, 276
Autoimmune disease, 147, 148
Awareness, 419

B
ß-lipoprotein, 275
Bone morphogenetic protein 2 (BMP2), 163
British sham-controlled apheresis study, 399

C
CAD, *see* Coronary artery disease (CAD)
Calcific aortic stenosis, 409
Calcific aortic valve disease (CAVD), 173, 275, 290
Calcific aortic valve stenosis (CAVS), 148, 149, 162
Candidate reference measurement procedure (cRMP), 338, 339
Cardiovascular disease (CVD), 135, 138, 148, 150, 162–166, 220, 221, 328
Cardiovascular mortality, 164
Cardiovascular outcomes trials, 354
Cardiovascular risk management, 349
Catabolism, 428
CD14++CD16+ intermediate monocyte subpopulation, 266
CD16+ monocytes, 264
CDC-Clinical Standardization Program (CSP), 342
Cell surface receptors
  kidney receptors, 130
  liver receptors, 127–130
  Lp(a)
    assembly, 127
    catabolism, 127
    structure, 125–127
  macrophages receptors, 131
Center for Disease Control (CDC), 303
Cerebral venous thrombosis, 191, 193
CETP inhibitors, 352
Cholesterol Reference Method Laboratory Network, 298
Cholesteryl ester transfer protein (CETP), 85, 352
Clot lysis, 195
Coagulation, 195–196
Commercial immunonephelometric assay, 334
Continuous ambulatory peritoneal dialysis (CAPD), 218–221
Copenhagen City Heart Study, 251, 253
Copenhagen cohorts, 255
Copenhagen General Population Study (CGPS), 242, 251, 347
Coronary artery disease, 135, 145, 148–151, 162, 165
Coronary calcium scores, 275
Coronary heart disease (CHD), 160, 165, 192, 199, 200
COVID-19, 152–153, 197, 198, 419, 422
CVD, *see* Cardiovascular disease (CVD)

D
Danger associated molecular patterns (DAMPs), 263
Deep vein thrombosis, 145
DELFIA, 429
Denka Seiken based assays, 307
The Denka Seiken reagents, 306
Direct assays, 235
Dresden Center for Extracorporeal Therapy, 386, 395

E
Electrospray differential ion mobility analysis, 310
Endogeneous fibrinolytic system, 190, 195
Enzyme-linked immunosorbent assay (ELISA), 304, 328, 429, 430
E-selectin, 162
European ancestry, 235
European Society of Cardiology (ESC)/ European Atherosclerosis Society (EAS) guidelines, 278, 347
European Union, 362
Evinacumab, 400
Evolocumab, 351
Extracorporeal therapy, 269, 381
Ezetimibe, 350

F
Familial hypercholesterolaemia (FH), 235, 276, 289, 432
Fibrinolysis, 141, 142, 145, 147, 152, 179–181

Fractional catabolic (or clearance) rate (FCR), 93, 382
Framingham Risk Score, 411

G
GalNac antisense, 353
GalNAc-modified ASOs, 370
Genetic Mendelian randomization studies, 347
Genetics
  KIV-2 VNTR, 63
  Lp(a) concentration, 55–61, 68
  Lp(a), structure of, 57
  LPA KIV-2 VNTR, 57–62, 67
  simple repeats or restriction site polymorphisms, 63
  SNPs, 63–66
German Lipoprotein Apheresis Registry, 388
Glomerular filtration rates (GFR), 209, 217, 218
Guidelines and position statements, 291

H
Healthcare system policies, 291
HEART UK consensus statement, 278, 382
Hemodialysis, 218–219
Hemorrhagic strokes, 195
Hepatocellular cancer, 140
Hepatocellular carcinoma (HCC), 142
High-density lipoprotein (HDL) metabolism, 130
High-density lipoprotein cholesterol (HDL-C), 351
HMG-CoA-reductase inhibitors, 24
Homeostasis, 189
HORIZON, 252
HPS2-THRIVE Study, 397
Human antisense oligonucleotides, 363
Human *LPA* gene, 58
Hyperlipoproteinemia, 392

I
Ile4399Met polymorphism, 145
Imaging modalities, 275
Inflammation, 135, 137–140, 142, 147–150, 152, 153, 191, 192, 196, 197, 200
INTERHEART study, 236
International Federation of Clinical Chemistry and Laboratory Medicine (IFCC), 300
International Lp workshop, 3
International Organization for Standardization (ISO), 300
Intracellular adhesion molecule (ICAM), 162
In-vitro diagnostic manufacturers, 299
In vivo kinetic studies, 216
Ionis pharmaceuticals, 360
IONIS-APO(a)-LRx trial, 371
Iron deficiency, 385
Ischemic stroke, 246
ISIS-APO(a)Rx complementary binding sites, 364

J
Joint Committee for Guides in Metrology (JCGM), 299
Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin (JUPITER) trial, 348, 349
Justification for the Use of Statins in the JUPITER study, 276

K
Kexin type 9 (PCSK9) inhibition, 166
Kidney disease
  early stages, 217, 218
  hemodialysis, 218–219
  high Lp(a) concentrations, 221, 222
  Lp(a), catabolism of, 221
  nephrotic syndrome, 217
  pathogenesis of, 222
  peritoneal dialysis, 218–219
  proteinuria, 217
  transplantation, 220
Kidney receptors, 130
Kringle 4 type 2 (KIV2), 231
Kringle domains, 9, 141
Kupffer cell-specific glycoprotein receptor, 48

L
LCAT deficiency (LCAT-D), 52
LDL-cholesterol, 409–415
LDL receptor (LDLR), 214, 215
LDLR-related protein 1 (LPR1), 118
Ld system, 40
Lipid-modifying agents, 351, 352

Lipoprotein
- accurate test results, 343
- age and sex and adjusted Cox proportional hazard ratios, 253
- ASCVD pathogenesis, 264, 265
- and ASCVD risk assessment, 276, 277
- assay methods, 285
- benefit of, 347, 348
- cardiovascular risk management, 335, 336
- characteristics and design of, 328–330
- clinical application, 289
- on clinical utility, 334
- contemporary immunoassay-based methods, 344
- in contemporary primary prevention studies, 252, 255
- in contemporary secondary prevention studies, 256, 258
- continuum of apoB-containing lipoprotein classes, 327, 328
- current therapies on, 348–353
- and CVD, 231, 233
  - aortic valve stenosis by, 244
  - concentration distribution, 242
  - elevated Lp(a) levels, 233, 234
  - familial hypercholesterolemia, 235
  - FOURIER trial, 233
  - ischemic stroke in, 246
  - primary and secondary prevention cohorts, 252
  - race and ethnicity on, 235, 236
- determinants of inaccuracy, 343
- dyslipidaemias, 232
- epidemiological and genetic evidence, 231
- experimental drugs
  - antisense oligonucleotides, 353
  - small interfering RNA, 354
- future perspectives, 258
- healthcare systems, 290
- and IgG and IgM autoantibodies, 262
- inflammatory mediators, 267, 268
- and innate immunity cells, 263, 264
- interim solutions, 342
- International Guidelines, 278
- interpretation, 289
- measurement for clinical purposes, 281, 282
- mechanisms of, 268
- method selection, 282, 284
- metrological traceability of, 326
- next generation reference measurement procedure, 340, 341
- next generation SI-traceable apo(a), 337, 338
- primary and secondary reference materials, 340
- regression dilution bias, 251
- risk thresholds for, 278
- safety and quality, 286, 287
- science and metrology, 326, 327
- scientific publications on, 252
- WHO reference material for, 342

Lipoprotein apheresis (LA)
- adverse effects and contraindications of, 385, 386
- apo(a) synthesis, 401
- cardiovascular events, 386–390, 395–398
- case reports, 391, 393
- in clinical laboratories, 429, 430
- effective therapy, 431
- efficiency of, 380–382
- hard CV endpoints, 430
- high Lp(a), 390, 420
- history of, 378, 380
- longevity and suppression, 426, 427
- metabolism, 427, 428
- oxidized phospholipids, 385
- pathophysiology, 428
- plasma Lp(a) levels, 432
- pros and cons of, 398, 399
- reveal Lp(a), 410–413
- screen for, 432
- TEST Lp(a), 413, 414

Lipoprotein receptor-related protein-1 (LRP1), 129

Lipoprotein(a) Foundation, 422

Lipoprotein(a)(Lp(a)), 40
- animal studies, 12
- antisense therapy, 24
- apo(a), 9, 10
- assembly, 127
- catabolism, 127
- CHD, 17, 18, 20, 21
- chemical composition, 44, 45
- definition, 207
- discovery of, 1–4
- epidemiological and genetic studies, 208
- evolution, 151, 152
- functional studies, 10, 11
- genetics, 12–16, 92
- historical review, 1
- HORIZON trial., 374
- isolation and characterization, 6–8
- LDL structure, impact of, 51–53
- Mendelian randomization, 18–21
- metabolism, 8, 9, 47
- non-genetic effects, 22
- plasma concentrations, 49–51
- preparation, 43, 44

protein structures, 45
PubMed search, 1
purification, 42
renal disease, 22, 23
structure, 57, 92, 125–127
transgenics, 12
type 2 diabetes mellitus, 22
Liposorber D, 395
Liver receptors, 127–130
Low density lipoprotein receptor-related protein 1 (LRP-1), 267
Low-density lipoprotein (LDL) cholesterol, 257
Low-density lipoprotein (LDL) particle, 92, 283
Low-molecular weight (LMW) isoforms, 208, 222
Lp(a) metabolism
clearance and catabolism, 82–84, 95, 96, 209
immune-histochemical studies, 209–211, 214
in vivo kinetic studies, 216
LDL receptor, 214, 215
concentration, 79–81
drug effects
apoB synthesis/secretion, 85
niacin, 85
PCSK9 inhibitors, 84
pelacarsen, 86
statins, 84
Volanesorsen, 86
LDL receptor, 80–82
lipid-regulating agents
apoB antisense oligonucleotides, 102
aspirin, 103
CETP inhibitors, 102
lomitapide, 103
niacin, 100
PCSK9 inhibitors, 100, 101
statins, 99, 100
THR agonists, 103
pharmacological interventions, 99
synthesis, assembly and secretion, 94–95, 208, 209
VLDL, LDL, and Lp(a) interconversion, 77–79
Lp(a)-mediated plaque instability, 197
Lp(a)-OxPhos-phospholipase pathway, 427
LPA-directed antisense oligonucleotides, 366
LPA gene, 57, 231
LPA genotyping, 286
LPA screening, 363
Lung cancer, 140
Lysophosphatidic acid, 268

M

Macrophage receptors, 131
Major adverse cardiovascular events (MACE), 257, 265, 350
Major adverse limb events (MALE), 431
Malignancies, impact of, 140
Mendelian randomization, 17–21, 23, 64, 195, 232, 251, 289, 431
Mendelian randomization-phenome-wide association approach, 194
Metastatic breast cancer, 140
Metrological traceability, 299, 326
Mighty Medic Group, 278
MOE-gapmers, 362
Monoclonal PCSK9 antibodies, 432
Monocyte/macrophage phenotype, 178
Monocytes, 263, 264
MTP inhibitor lomitapide, 352
MultiSELECt study, 390, 399
Myocardial infarctions (MIs), 161, 163, 165, 254

N

National Association of Statutory Health Insurance Physicians, 380
National Database for Cardiovascular Disease, 421
National Institute of Standards and Technology (NIST), 298
National Lipid Association (NLA), 278
Natural IgM antibodies, 262
Nephrotic syndrome (NS), 216, 217
Netherlands the Dutch External Quality Assessment (EQA-) organizer, 335
Neutrophil extracellular traps (NETs), 264
Neutrophil granulocytes, 264
Niacin, 85, 352
Non-apoB-directed therapies, 353
Nonsense mutation (R21X), 64, 65
Northwest Lipid Research Laboratories (NWRL), 304
Notch1-NFκB pathway, 178
Nuclear stress test, 412–413
Null allele, 13, 65, 66
Null mutations, 25
NWRL ELISA assay, 312

O

ODYSSEY OUTCOMES trial, 431
Olpasiran, 354, 431

Oral anticoagulants, 386
Ouchterlony test, 40
Ouchterlonys double diffusion, 1, 2
Oxidized LDLs (OxLDLs), 139, 262
Oxidized phospholipids (OxPL), 125, 135–137, 139, 140, 149, 151, 162, 174–176, 178, 179, 182, 196, 385, 427
Oxitope, 374

P
PAF acetyl hydrolase (PAF-AH), 427
Papillary thyroid cancer, 142
PCSK9 antibodies, 397
PCSK9 inhibitors, 24, 84, 350, 351, 400, 431
PCSK9-LDLR-Lp(a) axis, 117–119
Pearson correlation, 310
Pelacarsen, 353, 370, 397, 431
Pentanucleotide repeat (PNRP), 64
Peptide-calibrated reference measurement procedure, 340
Pericellular plasminogen activation, 144
Peripheral artery disease (PAD), 164
Peripheral vascular disease, 192
Peritoneal dialysis, 218–219
Personalized preventative medical care, 421
Phosphodiester (PO) bonds, 362
Phosphodiester (PO) linkages, 365
Phospholipase-A2, 427
Phosphorothioate (PS), 362
Plasma lipoprotein(a) concentrations
  apo(a) isoform size, 97, 98
  production rate vs fractional catabolic rate, 96–97
Plasminogen, 142–144, 147, 151, 152
Plasminogen activator inhibitor 1 (PAI-1), 189, 193
Platelets, 195–196
Pre-beta-lipoprotein, 22
Precision medicine, 421
Premature cardiovascular disease, 418
Production rate (PR), 382
Pro-inflammatory phenotype, 263
Proprotein convertase subtilisin kexin 9 (PCSK9)
  elevated plasma Lp(a) levels, 114, 116, 117
  LDL cholesterol-lowering drug target, 115, 116
  LDL-C metabolism, 113, 114
  PCSK9-LDLR-Lp(a) axis, 117–119
Prostate cancer, 142
Proteinuria, 217
Proteomic analysis, 267
Proteotypic peptides, 339

Q
Quality of life (QoL), 391

R
Radial immune-diffusion, 429
Radio-immuno assay (RIA), 429
Randomized controlled clinical trials (RCT), 416
Remnant receptor, 428
Reverse causation, 251
Reynold’s Risk Score, 411, 412
RNA-induced silencing complex (RISC), 354
Roche immunoassay, 340
Rocket electrophoresis, 429
Runt-related transcription factor 2 (RUNX2), 163

S
Scavenger receptor BI (SR-BI), 117
SDS-polyacrylamide gel electrophoresis, 7, 310
Seattle angina questionnaire (SAQ), 391
Selected reaction monitoring, 308
Selective thyroid hormone receptor (THR) agonists, 103
Sib pair linkage studies, 13
Single nucleotide polymorphisms (SNPs), 165, 232
Sinking pre-ß lipoprotein, 41, 42
SI units, 430
Six minute walk test (6MWT), 391
Small interfering RNA, 354
Southern blotting, 13, 14, 20
Stable isotopic tracer methodologies, 93, 94
Standardization
  clinical relevance of, 315, 316, 318
  current harmonization system, 305, 306
  history of, 303–305
  in clinical laboratory medicine, 298
  LC-MS/MS, 308–312
  measuring Lp(a), 301–303
  metrological traceability, 299, 301
Statins, 349, 350
Statistical analysis, 283
Stroke, 163–164, 409
Strong lysine-binding site (sLBS), 174, 175, 177

T
Thrombogenesis, 142
Thrombolysis, 179–182
Thrombosis, 142–145, 175, 179–182, 193–195
Tissue factor pathway inhibitor (TFPI), 144, 145, 189, 193
Tl system, 40
Triglyceride-rich lipoproteins, 287
Type 2 diabetes, 145–147

U
UK biobank, 254, 255

V
Valve interstitial cell phenotype, 179
Vascular cell adhesion molecule (VCAM)−1, 162
Vascular endothelial cell layer, 177
Vascular smooth muscle cells, 178
Vascular thrombosis, 409
Venous thromboembolism (VTE), 192, 194–196, 198, 200, 201
Venous thrombosis, 193–195, 200
Very-low-density lipoprotein (VLDL), 130, 428

W
Western blotting, 13, 23, 57–59, 64
Wilcoxon rank-sum test, 371
Women’s Heart Disease Data, 419
World health organization (WHO), 300
Wound healing, 147, 152

Y
Yeast artificial chromosomes (YACs), 12