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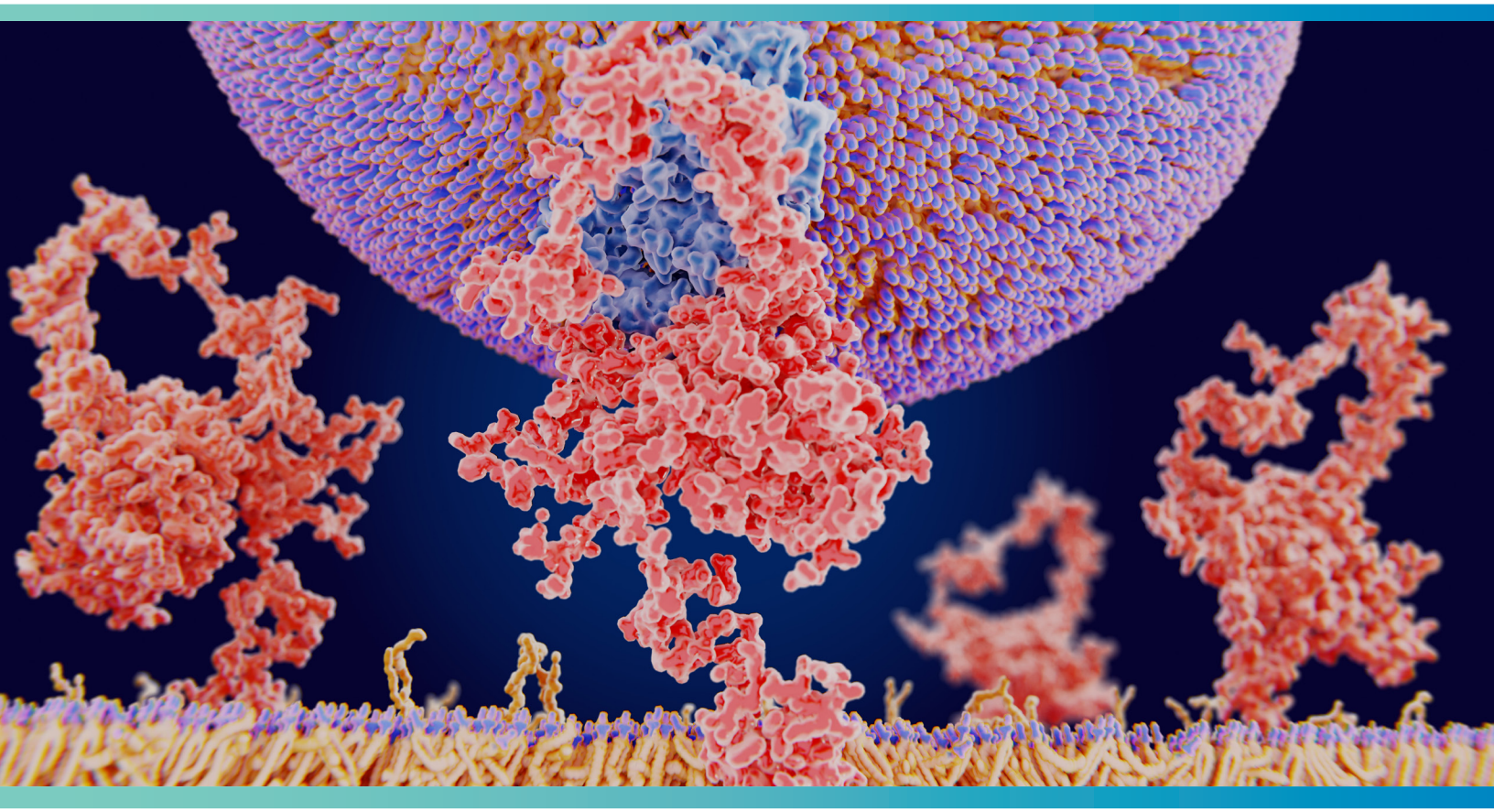

Direct Measurement of Lp(a)-Cholesterol Improves LDL-C Accuracy in Patients with Elevated Lp(a):

Analytical and Clinical Validation of a High-Throughput Assay

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Executive Summary

Accurate measurement of low-density lipoprotein cholesterol (LDL-C) is essential for cardiovascular risk assessment and treatment decisions. However, LDL-C values—regardless of the measurement methods—include cholesterol carried by lipoprotein(a) [Lp(a)], leading to significant overestimation of LDL-C in individuals with elevated Lp(a). This overestimation may misclassify risk, confound lipid-lowering therapy assessments, and obscure treatment efficacy in clinical trials.

Recent advances have enabled the direct quantification of Lp(a)-cholesterol [Lp(a)-C] using a high-throughput, immunocapture-based assay employing the murine monoclonal antibody LPA4 specific for apolipoprotein(a), followed by enzymatic cholesterol detection. This method is analytically validated, highly specific, and linear across the full range of Lp(a) concentrations. Application of this assay allows for the calculation of corrected LDL-C (LDL-C_{corr} = LDL-$C_{reported}$ − Lp(a)-C), which more accurately reflects true LDL particle cholesterol. The clinical implications are substantial. In patients with elevated Lp(a), the LDL-C_{corr} value may be 15-20 mg/dL lower than standard LDL-C, potentially affecting therapeutic thresholds and clinical trial outcomes.

Lp(a)-C and LDL-C_{corr} are emerging as important pharmacodynamic markers for Lp(a)-lowering therapies such as PCSK9 inhibitors and RNA-based agents directed to Lp(a). This novel assay—validated in a CLIA-certified setting—is positioned to support patient risk reclassification, therapeutic decision-making, and endpoint refinement in clinical trials.

Introduction and Background

The nomenclature of lipoproteins derives from the density gradient ultracentrifugation procedure used to separate lipoproteins according to their density ranges. The density is primarily determined by the lipid-to-protein ratio of each lipoprotein class. However, due to similar lipid/protein ratio, intermediate-density lipoprotein (IDL) and Lp(a) exhibit overlapping characteristics within the LDL density range. Therefore, LDL-C measured by ultracentrifugation, direct methods or common estimation formulas, also includes cholesterol from IDL and Lp(a)[1].

Lp(a) is an independent, genetically determined risk factor for atherosclerotic cardiovascular disease (ASCVD), contributing to atherogenesis, thrombosis, and inflammation[2]. While Lp(a) particle number, commonly used to assess risk, can be measured by sensitive and specific methods[3], Lp(a) also contains a substantial amount of cholesterol [Lp(a)-C], which directly affects LDL-C measurements. Lp(a)-C, in individuals with elevated Lp(a), results in significant overestimation of true LDL-C levels with potentially significant impact on clinical decision making.

To address this gap, Yeang et al[4] developed and validated a high-throughput, immunocapture-based assay to directly quantify Lp(a)-C. The assay demonstrated high specificity, linearity across a wide concentration range, and no detectable signal in plasma from individuals genetically deficient in Lp(a), confirming its precision.

The introduction of direct Lp(a)-C quantification enables a new LDL-C correction paradigm:

$$\textbf{LDL-}C_{corr} = \textbf{LDL-}C_{reported} - \textbf{Lp(a)-C}$$

This correction provides a more accurate representation of LDL-C considering that IDL-C is usually minimal in fasting samples. Importantly, this method resolves inconsistencies introduced by the assumption of a fixed percent cholesterol content per Lp(a) particle—an assumption invalidated by the demonstrated variability in Lp(a)-C mass per particle[5]. By isolating the Lp(a)-derived cholesterol fraction, this assay supports refined cardiovascular risk assessment, clearer interpretation of lipid-lowering trials, and improved clinical decision-making in patients with elevated Lp(a). It represents a key step toward personalized lipidology and the rational integration of emerging Lp(a)-targeted therapies.

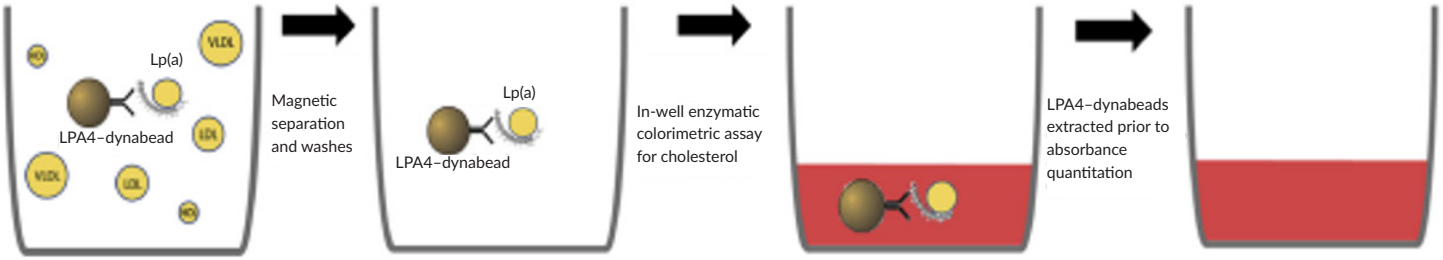
Analytical Methodology and Validation

The immunocapture assay involves several key steps:

1. **Immunocapture of Lp(a):** Plasma is incubated with magnetic beads coated with LPA4[6], a monoclonal antibody targeting apolipoprotein(a), selectively capturing Lp(a) particles while eliminating interference from cholesterol present from LDL, HDL, and VLDL.
2. **Magnetic separation and wash:** Beads with bound Lp(a) are magnetically separated, washed to remove unbound lipoproteins, thus enhancing specificity.
3. **Colorimetric cholesterol detection:** An enzymatic cholesterol reagent is added; the enzymatic reaction yields a colorimetric signal proportional to cholesterol content on captured Lp(a).
4. **Spectrophotometric quantification:** Absorbance is read at 500 nm (signal) and 700 nm (background), and Lp(a)-C concentrations are determined via a standard calibration curve.
5. **Standard curve and controls:** Cholesterol standards are used to quantify absolute Lp(a)-C levels in mg/dL
6. **Assay range:** The assay was shown to be linear across 0.78–40 mg/dL Lp(a)-C
7. **Application of LDL-C correction:** LDL-C_{corr} is calculated by subtracting measured Lp(a)-C from reported LDL-C values.

Figure 1. Methodology for Measuring Lp(a)-C.

Schematic of the Lp(a)-C assay. Lp(a) in plasma is affinity captured by LPA4-dynabeads and separated from other cholesterol carrying lipoproteins (depicted as yellow circles in the left panel) in each well by magnetic extraction and washes. Then, an enzymatic colorimetric cholesterol reagent is added to each well, generating a red color with intensity proportional to the amount of cholesterol present. Following a 5-min incubation period to ensure all cholesterol on Lp(a) has been processed, LPA4-dynabeads are extracted by magnet, and the absorbance at 500 (primary) and 700 nm (background) quantified.



The direct, specific, high-throughput assay quantifies Lp(a)-C using immunocapture (LpA4) and colorimetric detection. The method is linear at Lp(a) values up to 747 nmol/L and highly specific, with no cross-reactivity in Lp(a)-null samples[4]. It is high-throughput (96-well format) and rapid (about 1-hour total assay time).

There is a strong correlation between Lp(a)-C and Lp(a) particle concentration (r=0.76), reflecting the variability in Lp(a)-C among individual patients. In fact, it has been shown that the content of Lp(a)-C may range from ~6% to 57% of the total Lp(a) mass—with the higher percentages in patients with lower Lp(a) values and the lowest percentages in patients with elevated Lp(a) values[4].

Parameter	Value
Detection Method	Immunocapture (LPA4) + colorimetric assay
Dynamic Range	0.78 - 40.0 mg/dL
Linearity of Lp(a) values	Up to 747 nM Lp(a); $R^2 > 0.99$
Intra-assay CV	1.0 - 9.5%, depending on concentration
Lp(a)-C in Low Lp(a) Samples	0.0 - 3.0 mg/dL
Lp(a)-C in Elevated Lp(a) Samples	Up to 40.0 mg/dL
% Lp(a)-C of Lp(a) Mass	5.8% - 57.3%

Limitations of Prior Methods

Dahlén Formula

The Dahlén formula estimates Lp(a)-C by assuming a fixed proportion, typically 30%, of Lp(a) mass is cholesterol and subtracts this from LDL-C to derive a corrected value[5]. However, this method is fundamentally limited by its assumption of fixed composition, which fails to account for interindividual variability in Lp(a)-C content which can lead to substantial under- or overestimation of Lp(a)-C. In therapeutic settings, such as the pelacarsen trial[6], the Dahlén correction failed to reflect the significant reduction in Lp(a)-C seen with direct measurement, thus masking treatment effects. Moreover, it was never validated as a pharmacodynamic endpoint.

Densitometry-Based Methods

Densitometry-based methods for estimating Lp(a)-C involve separating lipoproteins via electrophoresis, staining for cholesterol, and quantifying the intensity of the presumed Lp(a) band using image analysis[7]. These techniques provided early evidence that Lp(a) contributes a significant amount of cholesterol—often estimated at 15–40% of Lp(a) mass. However, this method has limitations, including low specificity due to overlap between Lp(a) and LDL bands, poor reproducibility from semi-quantitative staining, and low sensitivity in quantitation of Lp(a)-C values in subjects with Lp(a) <30 mg/dL. This method is also labor-intensive, low-throughput, and less easily adapted to large pharmacological studies or for clinical use. Additionally, it lacks standardization and has not been validated against clinical outcomes or for monitoring therapeutic interventions.

Clinical Implications

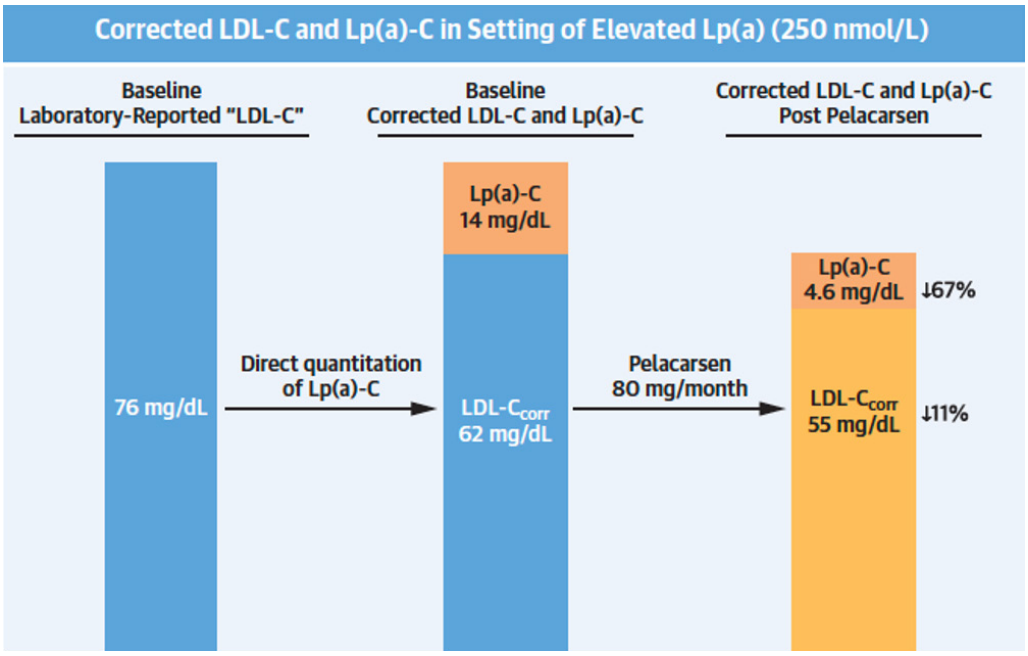
Direct quantification of Lp(a)-C improves risk assessment and enhances interpretation of lipid-lowering therapy in patients with elevated Lp(a). For example, this method was applied in a phase 2b randomized, double-blind, placebo-controlled, dose-ranging clinical trial designed to evaluate the effects of pelacarsen on Lp(a)-C and LDL-C_{corr} in patients with established cardiovascular disease and elevated Lp(a) levels[7]. A total of 286 subjects with elevated Lp(a) were randomized to receive placebo or pelacarsen administered subcutaneously. At baseline, the median Lp(a) concentrations across the groups ranged from 204 to 247 nmol/L, with corresponding median Lp(a)-C values ranging from 11.9 to 15.6 mg/dL. Laboratory-reported LDL-C values ranged from 68.5 to 89.5 mg/dL. However, LDL-C_{corr} was found to be on average 13 to 16 mg/dL lower than the standard LDL-reported values **(Figure 2)**.

The study demonstrated that pelacarsen significantly reduced directly measured Lp(a)-C in a robust, dose-dependent fashion, with reductions ranging from 29% to 67% compared with only a 2% reduction in the placebo group. While laboratory-reported LDL-C also declined modestly with pelacarsen, LDL-C_{corr} showed smaller changes, highlighting that the majority of the lipid-lowering effect was due to Lp(a)-C reduction rather than changes in LDL particle cholesterol.

Clinically, this study provides compelling evidence that directly measuring Lp(a)-C and deriving LDL-C_{corr}, is essential for accurate lipid assessment in patients with elevated Lp(a). For pharmaceutical development, especially for Lp(a)-targeting therapies in ongoing outcomes trials, using this method ensures that drug effects are not misattributed to LDL-C lowering, and it allows for precise stratification of lipid-related risk. This technique offers a valuable tool for future trials targeting Lp(a), as it can help clarify the distinct contributions of Lp(a)-C and LDL-related cholesterol to cardiovascular risk and may enhance interpretation of pharmacodynamic effects.

Figure 2. Lp(a)-C and LDL-Ccorr Before and After Pelacarsen Therapy.

A hypothetical patient is presented with a Lp(a) molar concentration of 250 nmol/L treated with pelacarsen 80 mg cumulative monthly. The baseline laboratory-reported LDL-C is 76 mg/dL. However, following direct measurement of the Lp(a)-C component of LDL the Lp(a)-C is 14 mg/dL and the corrected LDL-C is 62 mg/dL. Following treatment with pelacarsen, a 67% reduction in Lp(a)-C and 11% reduction in LDL-Ccorr is noted, achieving final concentrations of 55 mg/dL and 4.6 mg/dL, respectively. From Yeang et al7 with permission.



Potential Applications in Clinical Trials

1. **Patient Stratification:** Direct quantification of Lp(a)-C enables better stratification of cardiovascular risk, especially in patients with elevated Lp(a) or mixed dyslipidemia.
2. **Endpoint Refinement:** Lp(a)-C provides novel biomarkers that can serve as pharmacodynamic endpoints in trials of Lp(a)-lowering therapies.
3. **Mechanistic Insight:** Monitoring lipid composition on Lp(a) particles may uncover therapeutic effects beyond mass reduction, such as particle remodeling or inflammation attenuation.
4. **Therapy Selection:** Lp(a)-C changes could inform treatment decisions by identifying patients likely to benefit from targeted Lp(a)-lowering agents.
5. **Risk Reclassification:** Direct Lp(a)-C measurement and LDL-C_{corr} improve accuracy of primary and secondary prevention thresholds in lipid management guidelines.

Why Use the Lp(a)-C Assay at Medpace?

1. **CLIA Laboratory Implementation:** Performed in a CLIA- and CAP-accredited, laboratory, the assay ensures reliability, regulatory compliance, and consistency across global clinical trials.
2. **High Sensitivity and Specificity:** The Lp(a)-C assay offers superior analytical sensitivity compared to conventional Lp(a)-C assays, enabling precise quantification of cholesterol carried specifically on Lp(a) particles.
3. **Advanced Methodology:** Utilizes the LPA4 monoclonal antibody for immunocapture of Lp(a), followed by enzymatic colorimetric detection of cholesterol.
4. **High-Throughput and Scalable:** Optimized format with low intra- and inter-assay variability, making it ideal for large-scale pharmaceutical trials and biobanking studies.
5. **LDL-C Correction Capability**: Allows for accurate calculation of LDL-C_{corr}, improving risk stratification and lipid target assessment in individuals with elevated Lp(a).
6. **Clinical Relevance:** Valuable in studies of emerging Lp(a)-lowering therapies, where Lp(a)-C can serve as a pharmacodynamic marker distinct from other lipid and lipoprotein variables.
7. **Exclusive Research License:** Medpace central laboratories hold exclusive research rights from Megaron, Inc, using proven technology directly derived from and validated against the original gold-standard methods developed in Dr. Tsimikas' laboratory.
8. **Comprehensive Profiling:** The only reference lab offering both Lp(a)-C, LDL-C_{corr} with optional panels including Lp(a), apoB, OxPL-apoB and OxPL-apo(a) and inflammatory markers.

Full-Service Clinical Development

Medpace is a scientifically-driven, global, fullservice clinical contract research organization (CRO) providing Phase I-IV clinical development services to the biotechnology, pharmaceutical and medical device industries. Medpace's mission is to accelerate the global development of safe and effective medical therapeutics through its high-science and disciplined operating approach that leverages local regulatory and deep therapeutic expertise across all major areas including oncology, cardiology, metabolic disease, endocrinology, central nervous system and anti-viral and anti-infective.

About the Authors

Santica M. Marcovina, ScD, PhD, FAHA

Dr. Marcovina, is presently Sr. Director of Clinical Laboratory Sciences at Medpace Reference Laboratories. In this capacity, she oversees the validation and implementation of complex lipid and metabolic biomarkers, providing scientific and technical support to a team of laboratory scientists and medical technologists to ensure excellence in key parameters for endpoint and exploratory analyses in clinical trials. Before joining Medpace, she was for 30 years Research Professor of Medicine at the University of Washington and Director of the Northwest Lipid Research Laboratories where she has been the Principal Investigator in >60 NIH-funded clinical trials primarily in diabetes and its complications. Throughout her career, Dr. Marcovina's major field of research has been focused on Lp(a). Under NHLBI research grants, she developed high affinity monoclonal antibodies with well-defined epitope specificity on apo(a) kringle IV domains. These antibodies, and particularly one directed to a unique epitope present in apo(a) KIV9, were used to study the structure-function of Lp(a), and to demonstrate the impact of apo(a) KIV2 repeated domains on the accuracy of the immunochemical measurements of Lp(a). They were additionally used to develop an Lp(a) gold standard ELISA method and to develop a high-sensitivity agarose gel electrophoresis that first evidenced the existence of >35 apo(a) isoform size. Dr. Marcovina has published her work in major medical journals, including NEJM, Diabetes Care, EHJ, Nature, JAMA, JACC, Circulation, and has over 600 original papers, review articles, and book chapters.

Sotirios (Sam) Tsimikas, MD, FACC, FAHA

Dr. Tsimikas is a practicing Board-Certified Cardiologist and Professor of Medicine and the Director of Vascular Medicine at the University of California San Diego School of Medicine. His obtained his Medical Degree from the University of Massachusetts Medical School, Internal Medicine training at the University of Massachusetts Medical Center, and separate fellowships in Cardiovascular Medicine, Atherosclerosis and Molecular Medicine and Interventional Cardiology at the University of California San Diego (UCSD) Medical Center. He directs a basic and translational research laboratory focused on Lp(a) and their content of oxidized phospholipids as pro-inflammatory mediators of cardiovascular disease, with NIH-R01 funded research. He is the Founding Director of the Vascular Medicine Program within the Cardiovascular Medicine Division. He founded the UCSD "Lp(a) Clinic" as a novel paradigm to diagnose, manage and follow patients with elevated Lp(a). He has published his work in major medical journals, including NEJM, Lancet, Nature, Cell, JACC, Circulation, EHJ, and has over 400 original manuscripts, review articles and book chapters. In partnership with Dr Joseph Witztum, they defined the role of oxidized phospholipids (OxPLs) in atherosclerosis and vascular inflammation. In translational studies, they demonstrated that OxPL-apoB levels are elevated in individuals at risk for cardiovascular events and serve as both biomarkers and potential causal factors in atherogenesis. Through the development of specific assays and translational studies, they have established OxPL-apoB and OxPL-apo(a) as a mechanistically relevant and clinically actionable target in lipid-driven cardiovascular disease. The initial high-throughput Lp(a)-C was developed in Dr Tsimikas' laboratory.

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