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Lipoprotein(a) Concentration and Risks of Cardiovascular Disease and Diabetes

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ABSTRACT

BACKGROUND Lipoprotein(a) [Lp(a)] is a causal risk factor for cardiovascular diseases that has no established therapy. The attribute of Lp(a) that affects cardiovascular risk is not established. Low levels of Lp(a) have been associated with type 2 diabetes (T2D).

OBJECTIVES This study investigated whether cardiovascular risk is conferred by Lp(a) molar concentration or apolipoprotein(a) [apo(a)] size, and whether the relationship between Lp(a) and T2D risk is causal.

METHODS This was a case-control study of 143,087 Icelanders with genetic information, including 17,715 with coronary artery disease (CAD) and 8,734 with T2D. This study used measured and genetically imputed Lp(a) molar concentration, kringle IV type 2 (KIV-2) repeats (which determine apo(a) size), and a splice variant in *LPA* associated with small apo(a) but low Lp(a) molar concentration to disentangle the relationship between Lp(a) and cardiovascular risk. Loss-of-function homozygotes and other subjects genetically predicted to have low Lp(a) levels were evaluated to assess the relationship between Lp(a) and T2D.

RESULTS Lp(a) molar concentration was associated dose-dependently with CAD risk, peripheral artery disease, aortic valve stenosis, heart failure, and lifespan. Lp(a) molar concentration fully explained the Lp(a) association with CAD, and there was no residual association with apo(a) size. Homozygous carriers of loss-of-function mutations had little or no Lp(a) and increased the risk of T2D.

CONCLUSIONS Molar concentration is the attribute of Lp(a) that affects risk of cardiovascular diseases. Low Lp(a) concentration (bottom 10%) increases T2D risk. Pharmacologic reduction of Lp(a) concentration in the 20% of individuals with the greatest concentration down to the population median is predicted to decrease CAD risk without increasing T2D risk. (J Am Coll Cardiol 2019;74:2982-94) © 2019 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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Lipoprotein (a) [Lp(a)] is a risk factor for coronary artery disease (CAD) (1–6) and its related phenotypes (3,4,7–9). Mendelian randomization studies have provided strong evidence for a direct role of Lp(a) in their pathogenesis (3,5,8,10).

The Lp(a) particle consists of the apolipoprotein (a) [apo(a)] glycoprotein, which is encoded by the *LPA* gene and covalently bound to apolipoprotein B of modified low-density lipoprotein (LDL) (11). CAD risk has been associated with both Lp(a) molar concentration and apo(a) size, which are inversely correlated properties of the Lp(a) particle that are mostly genetically determined and highly heterogeneous in the general population. Both properties have been suggested to be independent risk factors for CAD (1–6,10–17).

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A common copy number polymorphism in the *LPA* gene determines apo(a) size through the number of copies of the kringle IV type 2 (KIV-2) protein domain of apo(a). This has been estimated to account for a large fraction of the variation in Lp(a) concentration, in part because of altered protein folding, transport, and secretion of larger isoforms (18). Lp(a) concentration has been estimated to be >90% genetically determined (19), and several associated variants have been reported, including loss-of-function mutations (20), variants associated with high Lp(a) levels and few KIV-2 repeats (2,21), and a splice variant G4925A that decreases Lp(a) molar concentration among individuals with few KIV-2 repeats, and therefore, small apo(a) isoforms (22). Individuals with few KIV-2 repeats and low Lp(a) molar concentration and carriers of G4925A deviate from the strong inverse relationship between apo(a) isoform size and Lp(a) molar concentration, and thus, provide an opportunity to disentangle whether Lp(a) molar concentration, apo(a) size, or both, affect CAD risk.

Increased risk of type 2 diabetes (T2D) has been associated with very low Lp(a) molar concentration and many KIV-2 repeats, but there is no correlation with T2D risk among individuals with higher molar concentration (23).

With Lp(a)–lowering drugs being developed, it is important to understand which attributes of Lp(a) best capture the cardiovascular risk and the consequences of Lp(a) lowering (e.g., T2D).

The aim of this study was to use Mendelian randomization to determine whether the effect of Lp(a) on CAD risk is through molar concentration or apo(a) isoform size and whether very low Lp(a) molar concentration increases T2D risk (**Figure 1**). We characterized sequence variants that affect Lp(a) molar concentration in the Icelandic population and tested the association of molar concentration and KIV-2 repeats with a multitude of cardiovascular traits.

ABBREVIATIONS AND ACRONYMS

apo(a) = apolipoprotein(a)
CAD = coronary artery disease
CI = confidence interval
HbA_{1c} = hemoglobin A_{1c}
HDL = high density lipoprotein
KIV-2 = kringle IV type 2
LDL = low density lipoprotein
Lp(a) = lipoprotein(a)
OR = odds ratio
SNP = single nucleotide polymorphism
T2D = type 2 diabetes

METHODS

The study was approved by the Data Protection Authority of Iceland and the National Bioethics Committee of Iceland. Enrollment of participants, the definitions of cardiovascular and cardiometabolic phenotypes, as well as information on blood lipid measurements, were previously described in detail (Online Appendix) (3,24,25). The aim of the study was to find associations between variations in the sequence of the genome and human phenotypes. The study started in 1996, and subjects with a broad range of phenotypes, their relatives, and control subjects have been recruited continuously since then. We assigned case status, including CAD, myocardial infarction, aortic valve stenosis, heart failure, atrial fibrillation, peripheral artery disease, and venous thromboembolism, based on the relevant International Classification of Diseases-9 and -10 codes for discharge diagnoses (1987 to 2018) or procedure codes (1982 to 2018), from Landspitali, The National University Hospital of Iceland, or from death registries (1972 to 2009) (Online Table 1). Cases with T2D were enrolled on the basis of 5 partially overlapping criteria: 1) confirmed diagnosis of participants in a long-term epidemiological study (26); 2) unrevised hospital diagnosis of T2D; 3) self-reported T2D; 4) use of oral diabetes medication; and 5) at least 1 measure of hemoglobin A_{1c} (HbA_{1c}) >6.5%. Diagnosis of type 1 diabetes was used as an exclusion criterion.

For each case–control analysis, the control group consisted of subjects free of the relevant disease.

MEASUREMENT OF Lp(a). We measured the molar concentration of Lp(a) in serum samples using a particle-enhanced turbidimetric immunoassay, Tina-quant Lipoprotein (a) Gen.2 (Roche, Basel, Switzerland)

employees of deCODE genetics, which is owned by the pharmaceutical company Amgen Inc. Amgen is currently developing Lp(a) lowering drugs aimed at decreasing coronary artery disease risk. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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run on a Cobas c 311 (Roche), a method that is independent of Lp(a) particle size and standardized to produce Lp(a) molar concentration (nmol/l) rather than density (mg/dl). We then imputed Lp(a) molar concentrations genetically into all chip-typed individuals based on haplotype sharing over chip single nucleotide polymorphisms (SNPs) (Online Appendix).

GENOTYPING AND IMPUTATION. Genotyping and imputation methods, as well as the association analysis method, were previously described (24,25,27). The sequence variants identified in subjects who underwent whole-genome sequencing were imputed into 151,677 Icelanders who had been genotyped with various Illumina (San Diego, California) SNP chips and their genotypes phased using long-range phasing (25,28) (Online Appendix).

MEASUREMENT OF KIV-2. We used whole-genome sequence data to measure KIV-2 (Online Appendix). KIV-2 repeat estimates were then refined and imputed based on haplotype sharing into all subjects with chip genotypes.

We also genotyped KIV-2 repeats by real-time polymerase chain reaction (TaqMan) in 6,640 subjects (Online Appendix). After using haplotype sharing to refine KIV-2 repeat measurements, the correlation between KIV-2 repeats in parent-offspring pairs was 0.52 using the TaqMan assay (658 pairs) compared with 0.67 when the whole-genome, sequencing-based method (4,374 pairs) was used. The correlation between KIV-2 repeats and serum Lp(a) particle number was also greater when we used the whole-genome sequencing methods ($R = -0.54$; $n = 6{,}068$) than when we used the TaqMan assay–based estimates ($R = -0.45$; $n = 6{,}543$). Together, these results indicated that the whole-genome sequencing-based and TaqMan methods were highly correlated, but that the whole-genome sequencing method provided a more accurate KIV-2 repeat estimate.

ASSOCIATION ANALYSIS. Linear and logistic regression were used to test the association of quantitative traits and case-control phenotypes, respectively, with sequence variants. The association testing was performed adjusted for sex and including sex-specific, first- and second-order terms for year of birth.

MENDELIAN RANDOMIZATION. Mendelian randomization is a method of using variation in the sequence to examine the causal effect of a nongenetic exposure on disease (29). In the context of this paper, the idea is to use sequence variants that affect Lp(a) molar concentration and KIV-2 repeats that are randomized at birth and are unaffected by Lp(a), to infer the causal relationship between these 2 measures and disease risk, in particular, CAD and T2D risk.

ASSOCIATION WITH LIFESPAN. To test for association with lifespan, we regressed lifespan against allele counts at each sequence variant. We calculated expected lifespan for subjects who were still alive and at least 65 years old based on their sex and year of birth. We assumed that death rates among alive subjects were the same as among subjects born before them. Subjects without an available death date who were <65 years or not registered as living in Iceland were excluded from the analysis. We did not exclude individuals based on cause of death (e.g., accidental, cancer, or other non-atherogenic cause of death). We performed 2 sets of analyses, based on individuals who lived to be at least 20 years old and those who lived to be at least 50 years old.

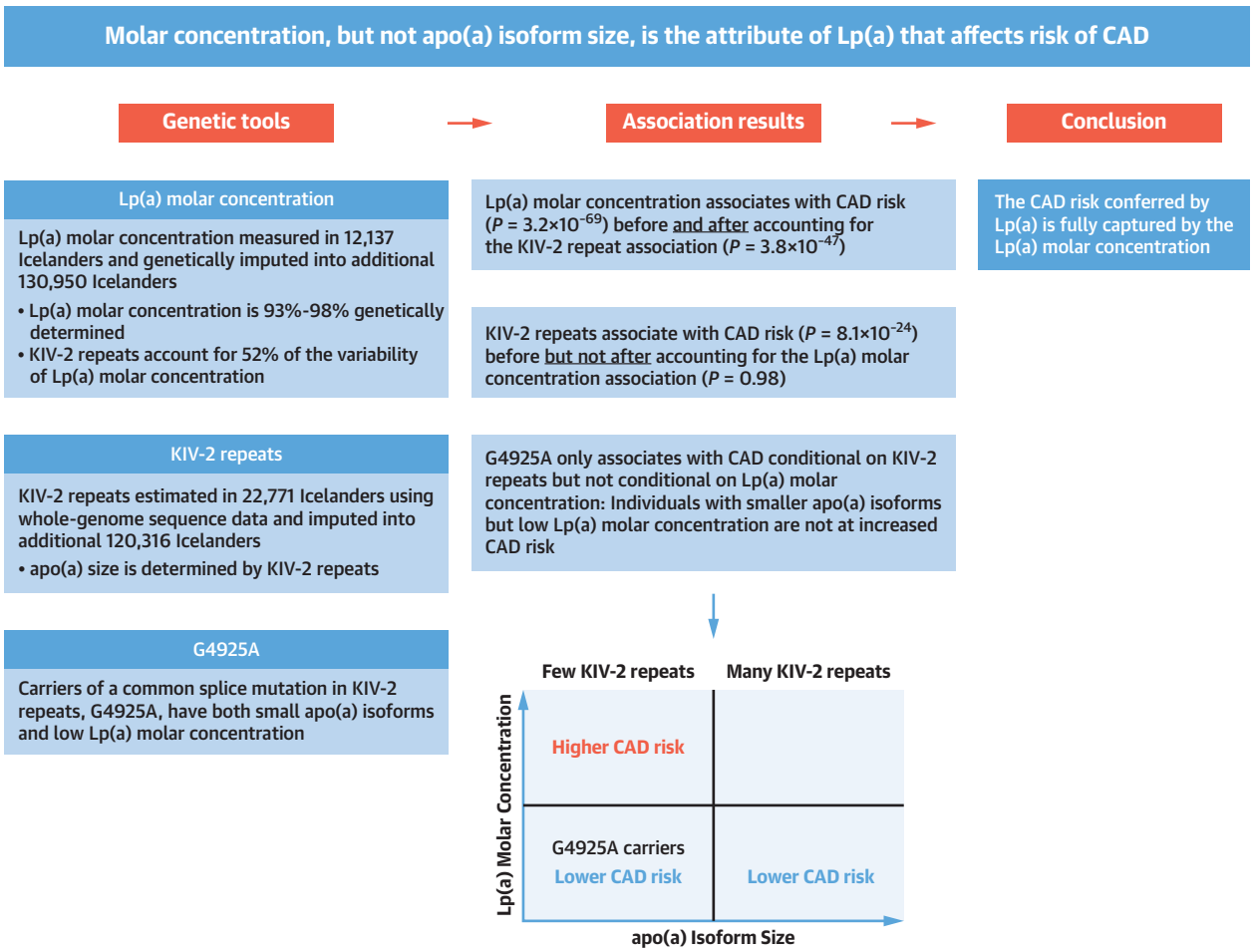
Lp(a) HERITABILITY ESTIMATION. We estimated Lp(a) heritability with 4 times the correlation between full siblings minus twice the correlation between parents and offspring (30).

RESULTS

DISTRIBUTION OF Lp(a) CONCENTRATION IN THE POPULATION. We measured Lp(a) molar concentration in 12,137 Icelanders using an immunoturbidimetric method that is insensitive to the size heterogeneity of apo(a) isoforms and is standardized according to units of molarity (nM). Online Figure 1A depicts the broad and skewed distribution of Lp(a) molar concentration in Iceland, consistent with that reported in other populations of European descent (19), ranging from zero to approximately 600 nM (first decile: 3.5 nM, median: 14.0 nM, and ninth decile: 122.9 nM).

SEQUENCE VARIANTS AFFECTING Lp(a) AND CAD. Sequence variants that associate with Lp(a) molar concentration or KIV-2 repeats are good Mendelian randomization tools for understanding the relationship between Lp(a) and cardiovascular disease risk. We used stepwise conditional analysis at *LPA,* based on variants detected by whole-genome sequencing of 15,220 Icelanders (24), to find the 3 SNPs that associated most strongly with high Lp(a) molar concentration and increased risk of CAD. One SNP was known (2,21), 1 was a refinement of a known signal (21), and 1 SNP was novel (Online Table 2, Online Appendix). Recently, a common splice mutation, G4925A (carrier frequency: 22.1%), in KIV-2 repeats was found to be associated with lower Lp(a) molar concentration and protection against CAD among subjects with apo(a) (22). We replicated the association of G4925A (allele frequency in Iceland: 13.3%) with low Lp(a) molar concentration, given the number of KIV-2 repeats (**Figure 2**).

FIGURE 1 Determination of the Relationship Between Lp(a) Molar Concentration, apo(a) Isoform Size and Cardiovascular Risk

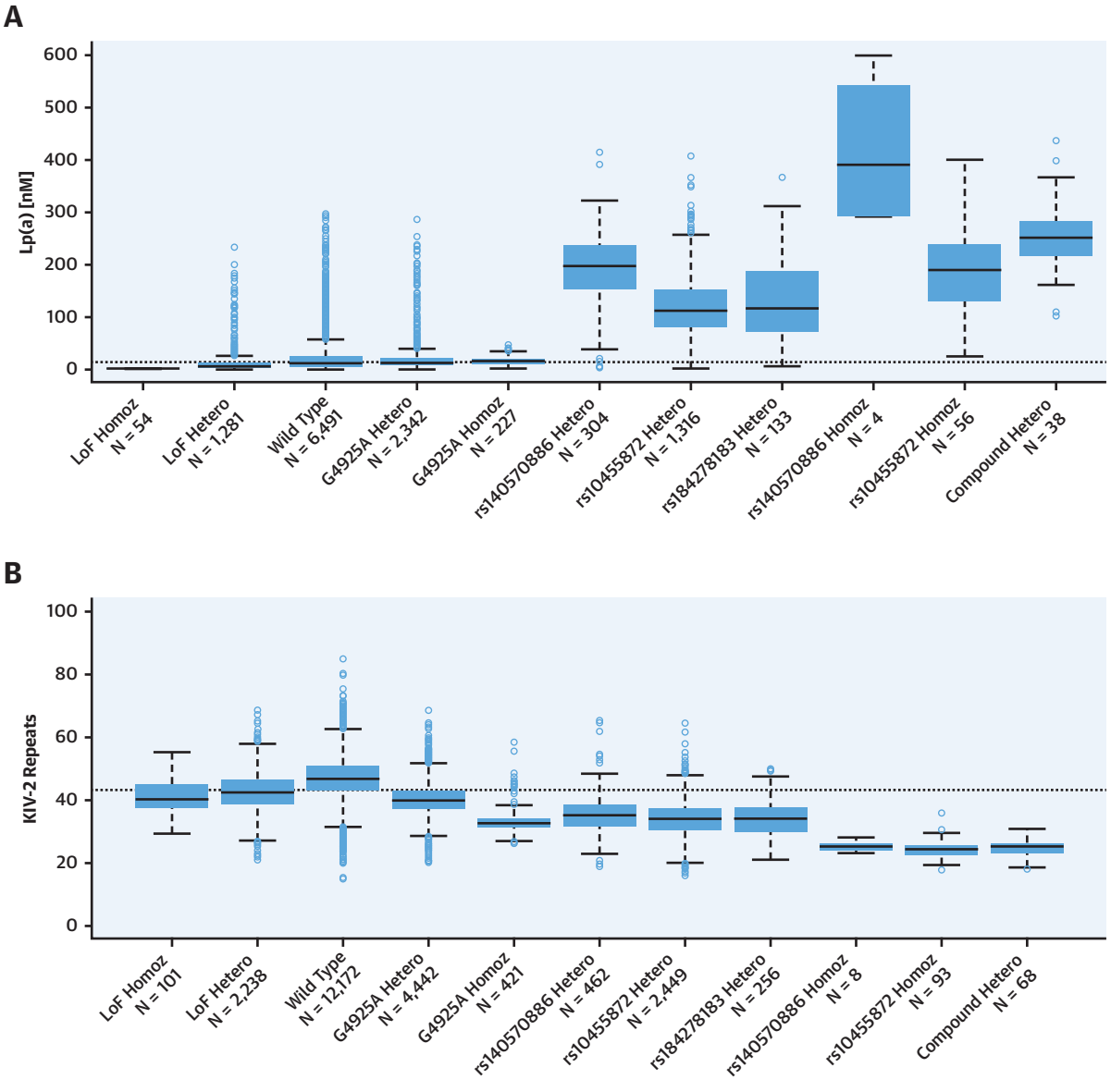


Higher risk of coronary artery disease (CAD) has been associated with higher lipoprotein(a) [Lp(a)] molar concentration and smaller apolipoprotein [apo(a)] isoforms. These are inversely correlated properties of the Lp(a) particle. We performed Mendelian randomization to determine whether the effect of Lp(a) on CAD risk is through molar concentration or apo(a) isoform size. We used measured and genetically imputed Lp(a) molar concentration, estimated kringle IV type 2 (KIV-2) repeats, and the G4925A splice mutation as tools, and tested for association with CAD in 17,715 Icelandic cases with CAD and 125,739 control subjects.

We identified 2 loss-of-function mutations in Iceland with a cumulative allele frequency of 6.2% (Online Appendix). Loss-of-function variants are useful tools for Mendelian randomization because homozygotes and compound heterozygotes for the loss-of-function mutations (1 in 260 individuals) always have very low Lp(a) molar concentration (median molar concentration: 2.0 nM, highest observed: 3.4 nM) (**Figure 2**). Thus, Lp(a) is absent or almost absent from the blood of these individuals, validating the loss-of-function annotation and the specificity of the Lp(a) assay.

ASSOCIATION OF Lp(a) MOLAR CONCENTRATION WITH CAD. We used long-range phasing (28) to infer haplotype sharing and to determine the relative contribution of the maternally and paternally derived alleles to the Lp(a) molar concentration and to impute Lp(a) molar concentration based on haplotype sharing into a set of 130,950 Icelanders, adding to the 12,137 Icelanders with directly measured molar concentration. This technique captured the multitude of sequence variants at *LPA* that affected Lp(a) molar concentration (31–33) without having to enumerate or model them explicitly. To evaluate the genetic imputation, we removed 1,202 subjects from the training set and found their imputation to be highly correlated with their measured values ($R^2 = 0.82$) (Online Figure 2). This accuracy indicated that Lp(a) molar concentration was almost completely determined by sequence variants at *LPA*. We

FIGURE 2 **Lp(a) Molar Concentration and KIV-2 Repeats by SNP Genotype and Allele**



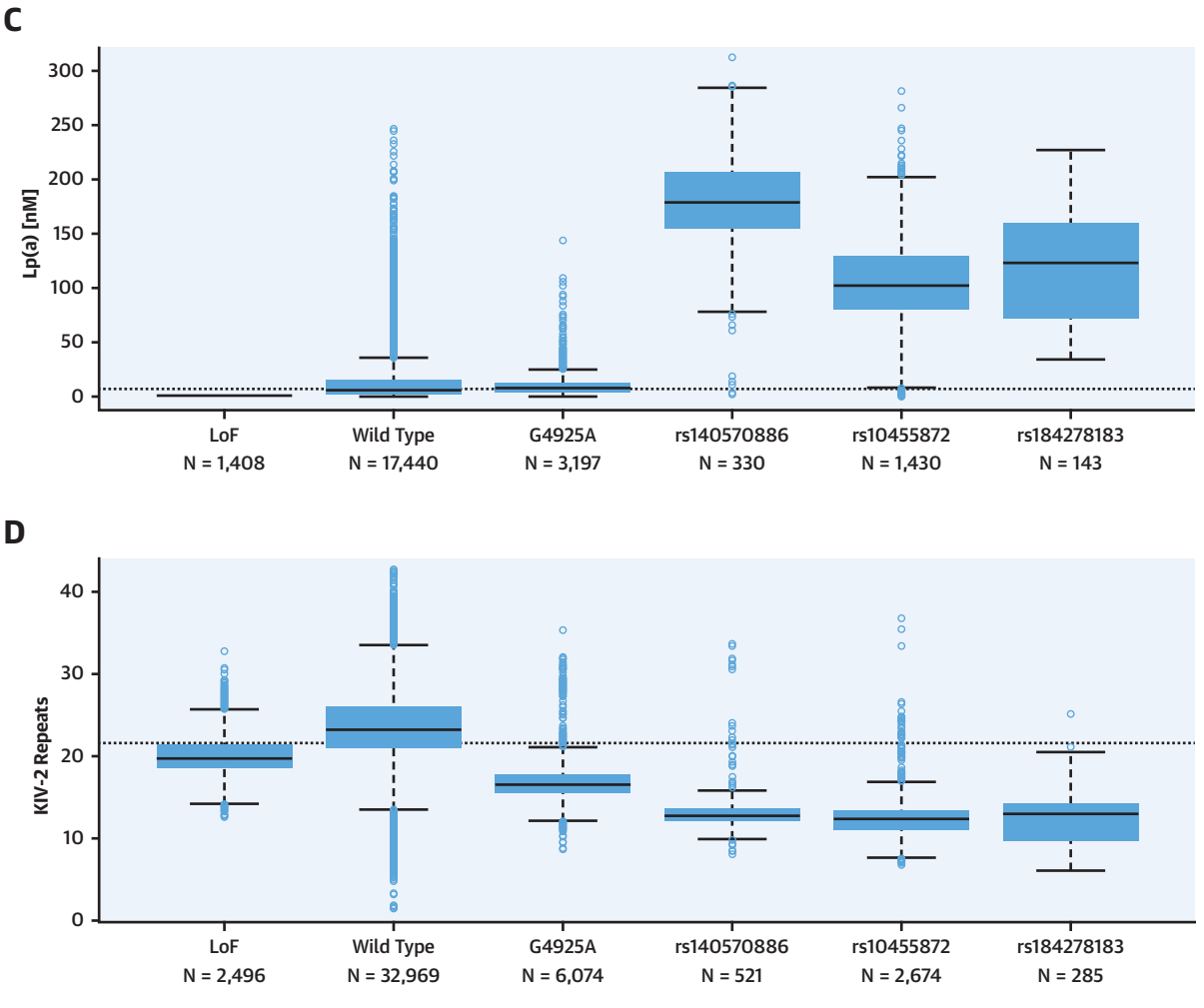
(A) Lp(a) molar concentration by single nucleotide polymorphism (SNP) genotype and **(C)** allele. KIV-2 repeats by **(B)** SNP genotype and **(D)** allele. Lp(a) molar concentration was measured in 12,137 subjects and KIV-2 repeats were measured using whole-genome sequencing in 22,771 subjects. The **bottom, middle, and top of the boxes** indicate the first, second (median), and third quartile, the **whiskers** indicate 1.5 times the interquartile range or the minimum/maximum values if the outlying values lie within this range. Observations outside 1.5 times the interquartile range indicated with points. Haplotype sharing was used to deconvolute the allelic effects of the SNPs (see Methods section). Hetero = heterozygous carriers; Homoz = homozygous carriers; LoF = loss-of-function variants; other abbreviations as in **Figure 1**.

Continued on the next page

estimated the heritability of Lp(a) molar concentration to be 0.88 based on the correlation between 2,893 parent-offspring ($r = 0.49$) and 2,943 sibling-pairs ($r = 0.46$), respectively.

Lp(a) molar concentration associated with the risk of CAD among 2,930 cases and 8,913 control subjects (odds ratio [OR]: 1.15 per 50 nM; $p < 0.0001$) (**Central Illustration**, Online Figure 3) with directly measured

FIGURE 2 Continued

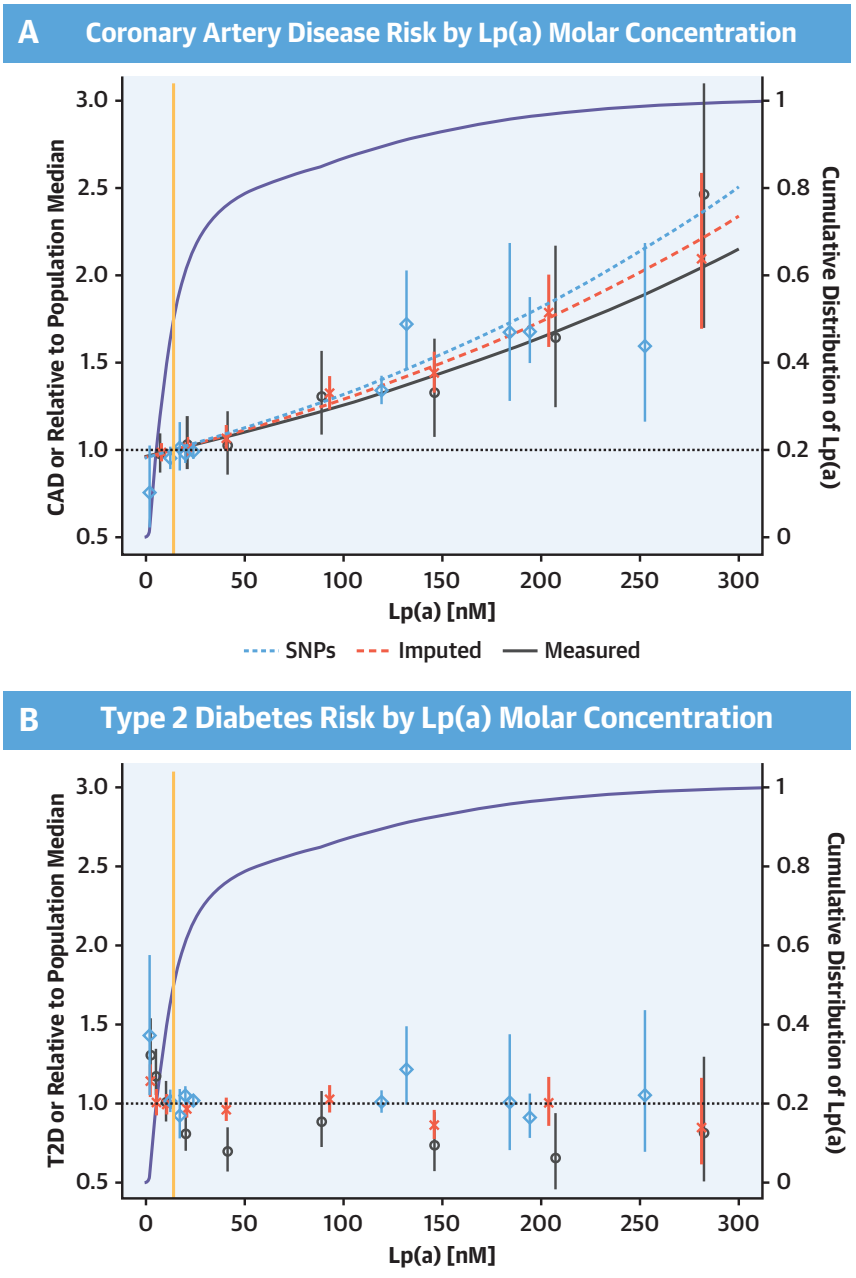


Lp(a) and similarly associated among 14,785 cases and 116,826 control subjects with genetically imputed Lp(a) concentrations (OR: 1.16 per 50 nM; $p < 0.0001$). Furthermore, the 3 SNPs with minor alleles that associated with increased Lp(a) molar concentration associated with CAD with a consistent effect (18,440 cases and 133,236 control subjects; OR: 1.17 per 50 nM; $p < 0.0001$). However, as expected, the association of the SNPs was completely accounted for by the genetically imputed Lp(a) molar concentration based on haplotype sharing over chip SNPs that did not include the Lp(a) increasing SNPs (p value after adjusting for Lp(a) molar concentration = 0.13). This indicated that the genetically imputed Lp(a) concentration captured all the association of the 3 SNPs in addition to other variants.

Our data suggested that the effect of sequence variants at *LPA* on CAD risk is proportional to their effect on Lp(a) molar concentration (**Central Illustration**). We predicted that 21.3%, 7.1%, and 1.3% of Icelanders have Lp(a) over 50 nM, 150 nM, and 250 nM, respectively, and that their CAD OR is 1.11, 1.50, and 2.01, respectively, relative to those at the population median of 14 nM (Online Table 3). Since these variants are all at *LPA*, their effect on Lp(a) molar concentration is most likely mediated through their effect on *LPA* expression.

Loss-of-function mutations in *LPA* were associated with protection against CAD risk (20). When we estimated the effects of the 2 loss-of-function mutations observed in the Icelandic population jointly with the 3 Lp(a)-increasing SNPs, we did not detect an association between the loss-of-function variants at the locus and CAD risk (Online Table 4). This discrepancy is explained by the fact that the previous study did not account for the well-established variants at the

CENTRAL ILLUSTRATION **CAD Risk and T2D Risk by Lp(a) Molar Concentration**



Gudbjartsson, D.F. et al. J Am Coll Cardiol. 2019;74(24):2982–94.

(A) Coronary artery disease (CAD) risk and **(B)** type 2 diabetes risk (T2D) by lipoprotein(a) [Lp(a)] molar concentration, expressed as odds ratios (OR) relative to the population median. The **whiskers** indicate 95% confidence intervals. The **yellow line** shows the median Lp(a) level. The **purple curve** shows the cumulative distribution of Lp(a) levels (see right y-axis for scale). The **blue diamonds** indicate Lp(a) single nucleotide polymorphism (SNP) genotype effects (see Figure 1). The **red x** indicates the CAD and T2D risk relative to the estimated risk of individuals with the population median Lp(a) molar concentration versus genetically imputed Lp(a) molar concentration split into bins at 0, 15, 30, 60, 120, 180, and 240 nM. The **black circles** indicate the same, but for measured Lp(a) molar concentration. **(A) Dotted blue**, **dashed red**, and **solid black lines** indicate the logistic regression fit of CAD risk as a function of Lp(a) measured by SNP effect, mean genetically imputed Lp(a) molar concentration, and mean measure Lp(a), respectively, adjusted for sex and first- and second-order terms for year of birth.

locus that increase Lp(a) concentration and CAD risk. Our association data excluded the possibility that the loss-of-function variants reduce the risk of CAD substantially compared with noncarriers with Lp(a) values close to the median.

ASSOCIATION WITH CARDIOVASCULAR AND METABOLIC PHENOTYPES AND OVERALL LIFESPAN. We tested a combined set of measured and genetically imputed Lp(a) molar concentrations for association with a range of cardiovascular and metabolic phenotypes, adjusting for year of birth and sex (**Table 1**, Online Table 5). Lp(a) molar concentration was associated with risk of various manifestations of CAD: myocardial infarction, CAD burden, left main coronary artery disease, and age of onset of CAD or myocardial infarction (**Table 1**). We also replicated previous reports of association of Lp(a) with aortic valve stenosis (8), heart failure (34), ischemic stroke (driven by large-vessel disease) (3,35), and peripheral vascular disease (36), but not venous thromboembolism (37) or chronic kidney disease (38).

Lp(a) molar concentration was associated with non–high-density lipoprotein (non-HDL) cholesterol levels (**Table 1**, Online Table 4). However, the CAD OR of approximately 1.15 per 50 nM Lp(a) increase in molar concentration ($p < 0.0001$) (**Table 1, Central Illustration**) was much greater than the predicted increase in risk based on the non-HDL cholesterol association (39). Therefore, the non-HDL association of the Lp(a) molar concentration cannot account for its strong CAD association.

We tested the 3 Lp(a) molar concentration–increasing SNPs for association with lifespan in subjects who lived to be at least 20 and 50 years old in a set that included the relatives of subjects who were chip genotyped. We found their lifespan to be 0.27 years shorter per 50 nM ($p < 0.0001$) and 0.22 years shorter per 50 nM ($p < 0.0001$), respectively (Online Table 6).

KIV-2 REPEATS, LP(A) MOLAR CONCENTRATION, AND CAD RISK. We estimated the number of KIV-2 repeats in 22,771 Icelanders using whole-genome sequence data and in 6,640 Icelanders using a quantitative polymerase chain reaction assay (TaqMan). We measured 1,336 subjects with both methods. Because the number of KIV-2 repeats is a property of the individual DNA sequence, we took advantage of haplotype sharing information derived from long-range phasing (28) to improve genotyping accuracy and to create phased alleles. The 2 methods gave consistent results ($R^2 = 0.78$). We used the whole-genome based method, which is superior to the TaqMan assay (Online Appendix). The distribution of phased KIV-2 repeats had a similar multimodal distribution, as previously reported (22).

Consistent with previous reports (40), we observed a clear inverse correlation between measured Lp(a) molar concentration and KIV-2 repeat estimates ($R = -0.52$; $n = 6,068$) (Online Figure 1E and 1F). The relationship was not simply a linear one, and the distribution of Lp(a) molar concentration for the fewer KIV-2 repeats was particularly broad. Some of the variability in Lp(a) molar concentration among subjects with few KIV-2 repeats was accounted for by the G4925A splice variant (22).

The relationship between the 6 *LPA* sequence variants [the 3 Lp(a) increasing SNPs, the 2 loss-of-function mutations, and the splice variant G4925A] and Lp(a) molar concentration and KIV-2 repeats at the genotype and haplotype level are shown in Online Figures 4 and 5.

In our set of 17,715 Icelandic cases with CAD and 125,739 control subjects with measured or genetically imputed Lp(a) molar concentration, the association of Lp(a) molar concentration with CAD risk was stronger than the association of KIV-2 repeats with CAD risk (**Table 1**). To compare the magnitude of effects in our study, we compared the increase in CAD risk per 50-nM increase of Lp(a) to the increase in CAD risk per 8.3 fewer KIV-2 repeats (both corresponded to 1.06 SDs). The estimated effect of a rise in Lp(a) molar concentration on CAD ORs was substantially greater than the effect of a comparable drop in the number of KIV-2 repeats (OR: 1.16; 95% confidence interval [CI]: 1.14 to 1.18 vs. OR: 1.09; 95% CI: 1.07 to 1.11). Moreover, after accounting for the association of Lp(a) molar concentration with CAD risk, the KIV-2 association was no longer significant ($p = 0.98$), whereas the Lp(a) association remained highly significant after accounting for KIV-2 repeats ($p < 0.0001$). This pattern was consistent for all cardiovascular diseases examined and for non-HDL and LDL cholesterol (**Table 1**).

Because carriers of G4925A produce Lp(a) (**Figure 2**) (22), it is not a loss-of-function mutation. Therefore, G4925A offers a second Mendelian randomization tool for testing whether Lp(a) molar concentration or apo(a) size is driving the CAD association, because carriers have both short apo(a) isoforms and Lp(a) molar concentrations that are close to that of individuals with long KIV-2 repeats (**Figures 1 and 2**). We replicated the association of G4925A with lower Lp(a) molar concentrations and less CAD risk among subjects with few KIV-2 repeats (Online Table 7). Similarly, G4925A was not associated with CAD risk on its own but was associated with CAD conditional on KIV-2 repeats. Conditional on Lp(a) molar concentration, G4925A was not associated with CAD risk at all.

TABLE 1 Association of Imputed Lp(a) Molar Concentration and KIV-2 Repeats With Biomarkers and Disease Phenotypes

		Lp(a) Molar Concentration	
	Cases/Controls	Effect (95% CI)	p Value
Case-control phenotypes			
CAD	17,715/125,739	1.16 (1.14 to 1.18)	<0.0001
Left main CAD	563/142,891	1.14 (1.06 to 1.23)	0.00031
Coronary artery bypass grafting	3,347/140,107	1.27 (1.24 to 1.31)	<0.0001
Myocardial infarction	9,575/133,879	1.18 (1.16 to 1.21)	<0.0001
Aortic valve stenosis	1,608/141,846	1.17 (1.12 to 1.22)	<0.0001
Heart failure	8,494/134,960	1.05 (1.02 to 1.07)	<0.0001
Atrial fibrillation	9,468/133,986	1.01 (0.99 to 1.03)	0.21
Ischemic stroke	4,209/139,245	1.03 (1.00 to 1.07)	0.023
Cardioembolic stroke	1,074/142,380	1.02 (0.96 to 1.08)	0.50
Small-vessel stroke	510/142,944	0.98 (0.90 to 1.06)	0.59
Large-vessel stroke	405/143,049	1.11 (1.01 to 1.21)	0.023
Peripheral artery disease	2,465/140,989	1.16 (1.12 to 1.21)	<0.0001
Venous thromboembolism	2,594/140,860	1.00 (0.96 to 1.04)	0.98
Chronic kidney disease	11,664 131,790	1.02 (1.00 to 1.04)	0.036
Quantitative phenotypes			
Coronary arteries 1 to 4 >50% stenosis*	7,347	0.06 (0.04 to 0.07)	<0.0001
CAD age at onset*	17,715	−0.22 (−0.31 to −0.12)	<0.0001
Age at myocardial infarction*	9,575	−0.14 (−0.26 to −0.02)	0.026
Total cholesterol†	94,920	0.06 (0.05 to 0.06)	<0.0001
HDL cholesterol†	88,644	0.01 (0.00 to 0.01)	0.012
LDL cholesterol†	82,854	0.06 (0.05 to 0.07)	<0.0001
Non-HDL cholesterol†	88,455	0.05 (0.04 to 0.06)	<0.0001
Triglycerides‡	77,186	−1 (−2 to −1)	<0.0001
Fasting glucose†	67,274	−0.01 (−0.01 to 0.00)	0.017
HbA_{1c}§	44,627	−0.01 (−0.01 to 0.00)	0.049

Effects for diseases are given in odds ratios (ORs). Effects are scaled to 50-nM increase in lipoprotein(a) [Lp(a)] concentration, and 95% confidence intervals (CIs) are given. Kringle IV type 2 (KIV-2) repeat effects have been inverted and scaled to correspond to 50 nM of a Lp(a) increase. Association testing for case-control phenotypes was performed with logistic regression and with linear regression for quantitative phenotypes, both adjusting for sex and year of birth. *Effects are SD. †Effects are in millimoles per liter. ‡Effects are in percentages. §Effects are in percentages of glycated hemoglobin.

CAD = coronary artery disease; HbA_{1c} = hemoglobin A_{1c}; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

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KIV-2 REPEATS AND HDL CHOLESTEROL AND TRIGLYCERIDES. Interestingly, the number of KIV-2 repeats associated negatively with HDL cholesterol and positively with triglycerides, even conditional on Lp(a) molar concentration (**Table 1**). Lp(a) concentration had weaker associations in the opposite direction with these traits (41), which were no longer significant after accounting for KIV-2 repeats. Because the *LPA* splice site variant G4925A affected the composition of apo(a) isoforms beyond what was predicted by KIV-2 (22), the variant should associate with HDL cholesterol and triglycerides given the KIV-2 repeat number. We found an association with HDL cholesterol ($p = 0.00052$) and triglycerides ($p = 0.0010$).

ASSOCIATION WITH T2D. The 10% of subjects with very low Lp(a) molar concentration (<3.5 nM) were reported to be at greater risk of T2D (23). We replicated this association (OR: 1.44; $p < 0.0001$) (**Central Illustration**, Online Figure 6, **Table 2**). However, T2D risk was independent of molar concentration in subjects with levels above the median. The top quintile of repeats was used as a tool in Mendelian randomization to show that the association between very low Lp(a) concentration and increased T2D risk was causal (42). We did not replicate this association (**Table 2**). Sequence variants associated with more KIV-2 repeats, but not with Lp(a) levels, were associated with increased risk of T2D in a small sample of 8,411 Danes (43). The interpretation was that many KIV-2 repeats were associated causally with increased risk of T2D but not low Lp(a) molar concentrations. However, we did not replicate this association (Online Table 8).

Loss-of-function homozygotes and subjects with very low genetically imputed Lp(a) molar

TABLE 1 Continued

KIV-2		Lp(a) Molar Concentration Adjusted for KIV-2		KIV-2 Adjusted for Lp(a) Molar Concentration	
Effect (95% CI)	p Value	Effect (95% CI)	p Value	Effect (95% CI)	p Value
1.09 (1.07 to 1.11)	<0.0001	1.16 (1.13 to 1.18)	<0.0001	1.00 (0.98 to 1.02)	0.98
1.07 (0.99 to 1.15)	0.11	1.16 (1.06 to 1.26)	0.0011	0.97 (0.88 to 1.07)	0.56
1.20 (1.16 to 1.24)	<0.0001	1.25 (1.21 to 1.30)	<0.0001	1.03 (0.99 to 1.08)	0.13
1.11 (1.09 to 1.14)	<0.0001	1.18 (1.15 to 1.21)	<0.0001	1.00 (0.98 to 1.03)	0.79
1.09 (1.04 to 1.14)	0.00028	1.18 (1.12 to 1.25)	<0.0001	0.98 (0.93 to 1.04)	0.47
1.03 (1.01 to 1.06)	0.0046	1.04 (1.01 to 1.07)	0.0048	1.01 (0.98 to 1.04)	0.46
1.02 (1.00 to 1.05)	0.025	1.00 (.97 to 1.03)	1.00	1.02 (1.00 to 1.05)	0.065
1.01 (0.98 to 1.04)	0.37	1.04 (1.00 to 1.08)	0.033	0.99 (.096 to 1.03)	0.64
1.02 (0.96 to 1.08)	0.57	1.02 (.95 to 1.09)	0.66	1.01 (0.94 to 1.08)	0.82
0.98 (0.90 to 1.06)	0.59	0.99 (0.89 to 1.09)	0.78	0.99 (0.89 to 1.09)	0.78
1.05 (0.96 to 1.15)	0.27	1.12 (1.00 to 1.24)	0.044	0.99 (0.88 to 1.10)	0.79
1.11 (1.07 to 1.15)	<0.0001	1.16 (1.11 to 1.21)	<0.0001	1.01 (0.96 to 1.06)	0.73
1.03 (0.99 to 1.07)	0.12	0.98 (0.93 to 1.02)	0.27	1.04 (1.00 to 1.09)	0.055
1.01 (0.99 to 1.03)	0.60	1.03 (1.00 to 1.05)	0.029	0.99 (0.97 to 1.01)	0.42
0.04 (0.03 to 0.06)	<0.0001	0.05 (0.03 to 0.07)	<0.0001	0.01 (−0.01 to 0.02)	0.25
−0.12 (−0.21 to −0.03)	0.012	−0.23 (−0.35 to −0.11)	<0.0001	0.03 (−0.03 to 0.08)	0.34
−0.09 (−0.20 to 0.02)	0.12	−0.14 (−0.28 to 0.01	0.061	0.00 (0.00 to 0.00)	0.50
0.03 (0.03 to 0.04)	<0.0001	0.06 (0.05 to 0.06)	<0.0001	0.00 (0.00 to 0.00)	0.47
0.02 (0.01 to 0.03)	<0.0001	−0.01 (−0.01 to 0.00)	0.066	0.02 (0.02 to 0.03)	<0.0001
0.04 (0.03 to 0.04)	<0.0001	0.06 (0.05 to 0.07)	<0.0001	0.00 (0.00 to 0.00)	0.34
0.02 (0.02 to 0.03)	<0.0001	0.05 (0.05 to 0.06)	<0.0001	−0.01 (−0.01 to 0.00)	0.025
−3 (−3 to −2)	<0.0001	0 (0 to 0)	0.41	−3 (−3 to −2)	<0.0001
0.00 (−0.01 to 0.00)	0.097	−0.01 (−0.01 to 0.00)	0.047	0.00 (0.00 to 0.00)	0.45
0.00 (0.00 to 0.00)	0.39	−0.01 (−0.01 to 0.00)	0.035	0.00 (0.00 to 0.01)	0.21

concentration (<3.5 nM) provide additional Mendelian randomization tools to explore the causality of the association between very low Lp(a) molar concentration and T2D (Table 2). The loss-of-function homozygosity associated with increased risk of T2D (OR: 1.45; p = 0.022) as did very low genetically imputed Lp(a) molar concentrations (OR: 1.16; p = 0.0012), which demonstrated a causal link between very low Lp(a) levels and T2D risk.

We investigated whether the association with T2D was driven by linkage disequilibrium with known T2D variants. A single SNP associated with T2D in the region around *LPA* (rs622217; published OR: 1.05) (44). This variant is only in weak linkage disequilibrium with the *LPA* loss-of-function variants (r^2 = 0.024) and conditioning on its effect did not affect the association of *LPA* loss-of-function homozygosity with T2D (conditional OR: 1.42; p = 0.030). Body mass

TABLE 2 Association Between Low Lp(a) Molar Concentration and T2D

	Cov%	No. T2D	No. Controls	T2D% With Covariate	T2D% Without Covariate	OR (95% CI)	p Value
KIV-2 top 20% vs. remainder	20	8,734	134,720	6.1	6.1	1.01 (0.95–1.06)	0.85
LoF homozygous	0.41	8,734	134,720	8.2	6.1	1.45 (1.05–1.99)	0.022
Measured Lp(a) <3.5 nM	10.1	1,548	10,295	16.4	12.7	1.44 (1.21–1.71)	<0.0001
Measured Lp(a) <3.5 nM - not LoF homozygous	9.8	1,537	10,253	16.2	12.7	1.42 (1.19–1.69)	0.00010
Imputed Lp(a) <3.5 nM*	7.4	7,186	124,425	6.3	5.4	1.17 (1.07–1.28)	0.00051
Imputed Lp(a) <3.5 nM* - not LoF homozygous	7.0	7,149	123,931	6.3	5.4	1.16 (1.06–1.27)	0.0012

*Does not overlap with individuals with measured Lp(a) molar concentration. The fraction of individuals with the covariate (Cov%) is shown, along with the number of cases, the number of controls, ORs, their 95% CIs, and p values are displayed. Association was performed with logistic regression, adjusting for sex and year of birth.

LoF = loss of function; T2D = type 2 diabetes; other abbreviations as in Table 1.

index is a major risk factor for T2D. Neither *LPA* loss-of-function homozygosity nor measured very low Lp(a) molar concentration associated with body mass index ($p > 0.26$), which demonstrated that the association with T2D is not driven by an association with body mass index.

DISCUSSION

Through large-scale estimation of Lp(a) molar concentration, KIV-2 repeats, and genotyping, we showed that the atherogenic effect of Lp(a) is conferred through the molar concentration and not apo(a) size, in contradiction to some of the existing literature (**Figure 1**). In particular, subjects with few KIV-2 repeats, and therefore, small apo(a) isoforms but low Lp(a) molar concentration were not at increased CAD risk. Similarly, carriers of the splice variant G4925A had short apo(a) isoforms but low Lp(a) molar concentration and were not at increased risk of CAD. This suggested that risk prediction based on Lp(a) should only depend on molar concentration and that treatment of Lp(a) should focus on lowering the molar concentration in subjects with high Lp(a) levels, regardless of the apo(a) size distribution. These findings were in line with analysis from the Bruneck study (45) ($n = 826$), which showed that Lp(a) levels improved cardiovascular disease risk prediction with no added value of apo(a) size.

We consistently found the same pattern for all cardiovascular diseases examined and for non-HDL and LDL cholesterol. Furthermore, because Lp(a) molar concentration was almost fully determined by sequence variants at *LPA* and was not substantially influenced by other factors (e.g., age, sex, other inherited factors, or the environment), the measured Lp(a) molar concentration captured most of the CAD risk associated with Lp(a). Consequently, both risk assessment and a therapeutic strategy can be based on measured Lp(a) molar concentration, regardless of the size of the apo(a) isoform and sequence variants that affect Lp(a) concentration.

Interestingly, apo(a) size appears to affect HDL cholesterol and triglyceride levels, while Lp(a) molar concentration does not. Neither HDL cholesterol nor triglyceride levels have been proven to have a causal effect on cardiovascular disease (39). Although these measures associate with CAD risk, their association is explained by their correlation with non-HDL cholesterol (39). This finding suggests that apo(a) size may have a biological function, although not conferring cardiovascular risk.

We replicated the association between very low measured Lp(a) molar concentration and increased T2D risk. In addition, we performed Mendelian randomization studies using loss-of-function variants and genetic imputation to show that this association is likely to be causal. We did not replicate the results of a previous study that used the top quintile of KIV-2 repeats as a Mendelian randomization tool (42), and we did not replicate a recent finding that suggested that the T2D risk association with Lp(a) was due to KIV-2 repeats rather than Lp(a) molar concentrations (43).

STUDY LIMITATIONS. Our study was limited to the Icelandic population. Follow-up in more European and in non-European populations is important. Imputation of genotypes and phenotypic prediction based on haplotype sharing was always inferior to direct measurement, although the imputations were shown to be sufficiently accurate. The measurement of Lp(a) molar concentration is not standardized in clinical practice; therefore, absolute thresholds and effect estimates may depend on the measurement platform (15). We noted that the median Lp(a) concentrations we observed were low relative to most other reports on Europeans, which is probably because of measurement heterogeneity rather than true differences between the populations.

CONCLUSIONS

Molar concentration was the attribute of Lp(a) that affected risk of CAD and other cardiovascular diseases. The association between the Lp(a) molar concentration and risk of CAD was dose-dependent and increased from an OR of 1.11 for those with Lp(a) levels at the 79th percentile (50 nM) to an OR of 2.01 for those with Lp(a) levels at the 99th percentile (250 nM), compared with those who had the Lp(a) population median level (14 nM). Lowering the Lp(a) levels of subjects with concentration above the 79th percentile (50 nM) to the population median of 14 nM could substantially reduce risk of cardiovascular disease and is not predicted to increase risk of T2D.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Based on Mendelian randomization in an Icelandic population, Lp(a) molar concentrations fully explained the association of Lp(a) with CAD, and there was no residual association with apo(a) particle size. Individuals with very low Lp(a) levels are at increased risk of developing T2D, indicating a causal link with absence of Lp(a).

TRANSLATIONAL OUTLOOK: Further studies are needed to verify the generalizability of these observations to other populations and to develop risk assessment and therapeutic strategies based on Lp(a) molar concentration rather than apo(a) isoform or sequence variants.

REFERENCES

1. Berg K, Dahlén G, Frick MH. Lp(a) lipoprotein and pre-beta1-lipoprotein in patients with coronary heart disease. Clin Genet 1974;6:230–5.

2. Nordestgaard BG, Chapman MJ, Ray K, et al. Lipoprotein(a) as a cardiovascular risk factor: current status. Eur Heart J 2010;31:2844–53.

3. Helgadottir A, Gretarsdottir S, Thorleifsson G, et al. Apolipoprotein(a) genetic sequence variants associated with systemic atherosclerosis and coronary atherosclerotic burden but not with venous thromboembolism. J Am Coll Cardiol 2012;60:722–9.

4. Kral BG, Kalyani RR, Yanek LR, et al. Relation of plasma lipoprotein(a) to subclinical coronary plaque volumes, three-vessel and left main coronary disease, and severe coronary stenoses in apparently healthy African-Americans with a family history of early-onset coronary artery disease. Am J Cardiol 2016;118:656–61.

5. Clarke R, Peden JF, Hopewell JC, et al. Genetic variants associated with Lp(a) lipoprotein level and coronary disease. N Engl J Med 2009;361:2518–28.

6. Kamstrup PR, Tybjaerg-Hansen A, Steffensen R, Nordestgaard BG. Genetically elevated lipoprotein(a) and increased risk of myocardial infarction. JAMA 2009;301:2331–9.

7. Kamstrup PR, Tybjærg-Hansen A, Nordestgaard BG. Elevated lipoprotein(a) and risk of aortic valve stenosis in the general population. J Am Coll Cardiol 2014;63:470–7.

8. Thanassoulis G, Campbell CY, Owens DS, et al. Genetic associations with valvular calcification and aortic stenosis. N Engl J Med 2013;368:503–12.

9. Thanassoulis G. Lipoprotein (a) in calcific aortic valve disease: from genomics to novel drug target for aortic stenosis. J Lipid Res 2016;57:917–24.

10. Kamstrup PR. Lipoprotein(a) and ischemic heart disease–a causal association? A review. Atherosclerosis 2010;211:15–23.

11. Schmidt K, Noureen A, Kronenberg F, Utermann G. Structure, function, and genetics of lipoprotein (a). J Lipid Res 2016;57:1339–59.

12. Paultre F, Pearson TA, Weil HF, et al. High levels of Lp(a) with a small apo(a) isoform are associated with coronary artery disease in African American and white men. Arterioscler Thromb Vasc Biol 2000;20:2619–24.

13. Kronenberg F, Utermann G. Lipoprotein(a): resurrected by genetics. J Intern Med 2013;273:6–30.

14. Kraft HG, Lingenhel A, Köchl S, et al. Apolipoprotein(a) kringle IV repeat number predicts risk for coronary heart disease. Arterioscler Thromb Vasc Biol 1996;16:713–9.

15. Marcovina SM, Albers JJ. Lipoprotein (a) measurements for clinical application. J Lipid Res 2016;57:526–37.

16. Kronenberg F. Human Genetics and the causal role of lipoprotein(a) for various diseases. Cardiovasc Drugs Ther 2016;30:87–100.

17. Saleheen D, Haycock PC, Zhao W, et al. Apolipoprotein(a) isoform size, lipoprotein(a) concentration, and coronary artery disease: a Mendelian randomisation analysis. Lancet Diabetes Endocrinol 2017;5:524–33.

18. Lanktree MB, Anand SS, Yusuf S, Hegele RA, SHARE Investigators. Comprehensive analysis of genomic variation in the LPA locus and its relationship to plasma lipoprotein(a) in South Asians, Chinese, and European Caucasians. Circ Cardiovasc Genet 2010;3:39–46.

19. Boerwinkle E, Leffert CC, Lin J, Lackner C, Chiesa G, Hobbs HH. Apolipoprotein(a) gene accounts for greater than 90% of the variation in plasma lipoprotein(a) concentrations. J Clin Invest 1992;90:52–60.

20. Lim ET, Würtz P, Havulinna AS, et al. Distribution and medical impact of loss-of-function variants in the Finnish founder population. PLoS Genet 2014;10:e1004494.

21. Kraft HG, Köchl S, Menzel HJ, Sandholzer C, Utermann G. The apolipoprotein (a) gene: a transcribed hypervariable locus controlling plasma lipoprotein (a) concentration. Hum Genet 1992;90:220–30.

22. Coassin S, Erhart G, Weissensteiner H, et al. A novel but frequent variant in LPA KIV-2 is associated with a pronounced Lp(a) and cardiovascular risk reduction. Eur Heart J 2017;38:1823–31.

23. Mora S, Kamstrup PR, Rifai N, Nordestgaard BG, Buring JE, Ridker PM. Lipoprotein(a) and risk of type 2 diabetes. Clin Chem 2010;56:1252–60.

24. Jónsson H, Sulem P, Kehr B, et al. Whole genome characterization of sequence diversity of 15,220 Icelanders. Sci Data 2017;4:170115.

25. Gudbjartsson DF, Helgason H, Gudjonsson SA, et al. Large-scale whole-genome sequencing of the Icelandic population. Nat Genet 2015;47:435–44.

26. Reynisdottir I, Thorleifsson G, Benediktsson R, et al. Localization of a susceptibility gene for type 2 diabetes to chromosome 5q34-q35.2. Am J Hum Genet 2003;73:323–35.

27. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 2010;20:1297–303.

28. Kong A, Masson G, Frigge ML, et al. Detection of sharing by descent, long-range phasing and haplotype imputation. Nat Genet 2008;40:1068–75.

29. Katan MB. Apolipoprotein E isoforms, serum cholesterol, and cancer. Lancet 1986;1:507–8.

30. Suzuki DT, Griffiths AJ, Miller JH, Lewontin RC. An Introduction to Genetic Analysis. New York: WH Freeman and Company, 1986.

31. Lu W, Cheng Y-C, Chen K, et al. Evidence for several independent genetic variants affecting lipoprotein (a) cholesterol levels. Hum Mol Genet 2015;24:2390–400.

32. Mack S, Coassin S, Rueedi R, et al. A genome-wide association meta-analysis on lipoprotein(a) concentrations adjusted for apolipoprotein(a) isoforms. J Lipid Res 2017;58:1834–44.

33. Kettunen J, Demirkan A, Würtz P, et al. Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA. Nat Commun 2016;7:11122.

34. Kamstrup PR, Nordestgaard BG. Elevated lipoprotein(a) levels, LPA Risk genotypes, and increased risk of heart failure in the general population. J Am Coll Cardiol HF 2016;4:78–87.

35. Erqou S, Kaptoge S, Perry PL, et al. Lipoprotein(a) concentration and the risk of coronary

heart disease, stroke, and nonvascular mortality. JAMA 2009;302:412–23.

36. Catalano M, Cortelazzo A, Yilmaz Y, Perilli E, Carzaniga G, Emanuele E. The LPA gene C93T polymorphism influences plasma lipoprotein(a) levels and is independently associated with susceptibility to peripheral arterial disease. Clin Chim Acta 2008;387:109–12.

37. Sticchi E, Magi A, Kamstrup PR, et al. Apolipoprotein(a) kringle-iv type 2 copy number variation is associated with venous thromboembolism. PloS One 2016;11:e0149427.

38. Emdin CA, Khera AV, Natarajan P, et al. Phenotypic characterization of genetically lowered human lipoprotein(a) levels. J Am Coll Cardiol 2016;68:2761–72.

39. Helgadottir A, Gretarsdottir S, Thorleifsson G, et al. Variants with large effects on blood lipids and the role of cholesterol and triglycerides in coronary disease. Nat Genet 2016;48:634–9.

40. Sandholzer C, Hallman DM, Saha N, et al. Effects of the apolipoprotein(a) size polymorphism on the lipoprotein(a) concentration in 7 ethnic groups. Hum Genet 1991;86:607–14.

41. Contois JH, Lammi-Keefe CJ, Vogel S, et al. Plasma lipoprotein (a) distribution in the Framingham Offspring Study as determined with a commercially available immunoturbidimetric assay. Clin Chim Acta 1996;253:21–35.

42. Kamstrup PR, Nordestgaard BG. Lipoprotein(a) concentrations, isoform size, and risk of type 2 diabetes: a Mendelian randomisation study. Lancet Diabetes Endocrinol 2013;1:220–7.

43. Tolbus A, Mortensen MB, Nielsen SF, Kamstrup PR, Bojesen SE, Nordestgaard BG. Kringle IV type 2, not low lipoprotein(a), as a cause of diabetes: a novel genetic approach using SNPs associated selectively with lipoprotein(a) concentrations or with kringle iv type 2 repeats. Clin Chem 2017;63:1866–76.

44. Zhao W, Rasheed A, Tikkanen E, et al. Identification of new susceptibility loci for type 2 diabetes and shared etiological pathways with coronary heart disease. Nat Genet 2017;49:1450–7.

45. Willeit P, Kiechl S, Kronenberg F, et al. Discrimination and net reclassification of cardiovascular risk with lipoprotein(a): prospective 15-year outcomes in the Bruneck Study. J Am Coll Cardiol 2014;64:851–60.

KEY WORDS coronary artery disease, genetics, Lp(a), Mendelian randomization, type 2 diabetes

APPENDIX For an expanded Methods section and supplemental figures and tables, please see the online version of this paper.