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CME

Extreme Lipoprotein(a) Levels and Improved Cardiovascular Risk Prediction

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Extreme Lipoprotein(a) Levels and Improved Cardiovascular Risk Prediction

Objectives	The study tested whether extreme lipoprotein(a) levels and/or corresponding <i>LPA</i> risk genotypes improve myocardial infarction (MI) and coronary heart disease (CHD) risk prediction beyond conventional risk factors.
Background	Elevated lipoprotein(a) levels cause MI and CHD. Levels are primarily determined by variation in the <i>LPA</i> gene.
Methods	We followed 8,720 Danish participants in a general population study from 1991 to 1994 through 2011 without losses to follow-up. During this period, 730 and 1,683 first-time MI and CHD events occurred. Using predefined cutpoints for extreme lipoprotein(a) levels and/or corresponding <i>LPA</i> risk genotypes (kringle IV type 2 [KIV-2]) repeat polymorphism, rs3798220, and rs10455872 single nucleotide polymorphisms), we calculated net reclassification indices from <10% to 10% to 19.9% to ≥20% absolute 10-year MI and CHD risk.
Results	For individuals with lipoprotein(a) levels ≥80th percentile (≥47 mg/dl), 23% ($p < 0.001$) of MI events and 12% ($p < 0.001$) of CHD events were reclassified correctly, while no events were reclassified incorrectly for either endpoint. As some incorrect reclassification of individuals with no events occurred, addition of lipoprotein(a) levels ≥80th percentile overall yielded net reclassification indices of +16% (95% confidence interval: 8% to 24%) and +3% (−1% to 8%) for MI and CHD, respectively. Corresponding net reclassification indices for number of KIV-2 repeats ≤21st percentile were +12% (5% to 19%) and +4% (0% to 8%), for rs3798220 carrier status +15% (−14% to 44%) and +10% (−10% to 30%), and for rs10455872 carrier status +16% (6% to 26%) and +2% (−1% to 6%). Considering only individuals at 10% to 19.9% absolute 10-year MI and CHD risk, addition of extreme lipoprotein(a) levels or corresponding <i>LPA</i> risk genotypes improved risk prediction even further.
Conclusions	Extreme lipoprotein(a) levels or corresponding <i>LPA</i> KIV-2/rs10455872 risk genotypes substantially improved MI and CHD risk prediction. (J Am Coll Cardiol 2013;61:1146–56) © 2013 by the American College of Cardiology Foundation

Elevated lipoprotein(a) levels are considered a causal risk factor for coronary heart disease (CHD) (1,2). Epidemiologic studies including meta-analyses have amply demonstrated a robust and specific association of elevated lipoprotein(a) levels with increased risk of CHD (3,4). Of note, meta-analyses risk estimates may represent minimal estimates as early negative studies using questionable lipoprotein(a) measurements are included. We, and others, have previously reported that in particular extreme levels associate with a markedly increased risk (5–7), and recent studies of *LPA* gene variants strongly support that the association is causal (8,9). However, data on extreme lipoprotein(a) levels and/or corresponding *LPA* risk genotypes are not included in current cardiovascular risk prediction tools (2,10,11). Thus, individuals at high risk of CHD due to extreme lipoprotein(a) levels may remain unidentified.

Recently, a study combining data from 24 prospective studies reported only slight improvements in cardiovascular disease risk prediction when adding lipoprotein(a) levels to conventional risk factors (12). However, even if lipoprotein(a), due to its skewed concentration distribution with a tail toward extremely high levels, will not improve risk prediction markedly in the average person with low to moderate levels, lipoprotein(a) measurements has the potential to substantially improve risk prediction in a person with extreme levels. This corresponds to the common sense approach of most doctors to only notice and take action upon lipoprotein(a) levels in the extreme range. The present paper follows this common sense approach using recommended prediction tools (13,14).

Lipoprotein(a) consists of a cholesterol-laden low-density lipoprotein (LDL)-like particle bound to a plasminogen-like glycoprotein, apolipoprotein(a) (15). The dual structure suggests that lipoprotein(a) may be able to contribute to both atherosclerosis and thrombosis, and recent human genetic data support a role for lipoprotein(a) in atherosclerotic stenosis in particular (16). Plasma lipoprotein(a) levels may vary up to a thousand-fold between healthy individuals and are primarily determined by variation in the *LPA* gene coding for apolipoprotein(a) (15). Most influential is the so-called kringle IV type 2 (KIV-2) repeat polymorphism defined by a 5.6 kb large repeat that may occur between 2 and >40 times per allele, determining the size of the expressed apolipoprotein(a) protein, the size of which correlates inversely with plasma lipoprotein(a) levels (15). Some studies have indicated that in particular small isoforms are harmful to an extent not fully explained by the concomitantly elevated lipoprotein(a) levels (7,17,18). We have previously shown that a lower number of KIV-2 repeats associates with increased risk of myocardial infarction (MI) (8), and in addition, carriers of 2 *LPA* rare allele single nucleotide polymorphisms (SNPs) (rs3798220 and rs10455872) tagging the KIV-2 repeat polymorphism, have also been reported to be at increased risk of CHD (9).

In the present study, we tested the hypothesis that addition of information on extreme lipoprotein(a) levels and/or corresponding *LPA* risk genotypes may improve MI and CHD risk prediction based on conventional risk factors. For this purpose, we used a prospective study of the Danish general population, the CCHS (Copenhagen City Heart Study), with baseline information on conventional risk

**Abbreviations
and Acronyms**

- CHD** = coronary heart disease
- CI** = confidence interval
- HDL** = high-density lipoprotein
- IDI** = integrated discrimination improvement
- KIV-2** = kringle IV type 2
- LDL** = low-density lipoprotein
- MI** = myocardial infarction
- NRI** = net reclassification index
- SNP** = single nucleotide polymorphism

factors, lipoprotein(a) levels, *LPA* KIV-2 repeat genotypes, and *LPA* rs3798220 and rs10455872 SNPs.

Methods

Participants. The CCHS was initiated in 1976 to 1978 with follow-up examinations in 1981 to 1983, 1991 to 1994, and 2001 to 2003 (19). Participants were randomly selected within age and sex strata from the Copenhagen Civil Registration System to reflect the general population of Copenhagen aged 20 to 80+ years. For the present study, we included CCHS 1991 to 1994 examination participants (16,563

invited, 61% response rate) of Danish descent with no prior history of CHD, who had lipoprotein(a) measurements performed shortly after sampling, and who had a blood sample available for DNA analyses, as well as complete information on conventional risk factors (N = 8,720) (19). Approximately half of the included participants also had a lipoprotein(a) measurement performed in 2001 to 2003, allowing correction for regression dilution bias (20).

Examinations included a self-administered questionnaire and a physical examination. Smokers were active smokers, and diabetes mellitus was defined as self-reported disease, use of insulin or oral hypoglycemic drugs, and/or a nonfasting plasma glucose of >11 mmol/l. We followed all individuals from baseline in 1991 to 1994 and censored at the occurrence of CHD (including MI), death, or May 2011, whichever came first. Follow-up was 100% complete, that is, we did not lose track of a single individual during follow-up.

Information on diagnoses of MI and CHD (International Classification of Diseases-8th edition, codes 410 and codes 410-414, respectively, and 10th edition, codes I21-I22 and I20-I25, respectively) was collected and verified by reviewing hospital admissions and diagnoses entered in the national Danish Patient Registry, causes of death entered in the national Danish Causes of Death Registry, and medical records from hospitals and general practitioners.

The study was approved by Herlev Hospital and a Danish ethical committee (No. KF-100.2039/91) and was conducted according to the Declaration of Helsinki. All participants gave written informed consent.

Laboratory analyses. Lipoprotein(a) total mass was measured using an in-house assay, as described previously(6), and as detailed in the Online Appendix and Online Figure 1. Enzymatic assays were used on fresh samples to measure plasma levels of total cholesterol and high-density lipoprotein (HDL) cholesterol. The *LPA* KIV-2 repeat polymorphism was genotyped by real-time polymerase chain reac-

tion analysis yielding an estimate of the total number of KIV-2 repeats on both alleles, as described previously(8).

Genotyping for *LPA* rs3798220 and rs10455872 SNPs was performed by TaqMan analysis. Genotype distributions did not differ from those predicted by the Hardy-Weinberg equilibrium.

Statistical analyses. A priori we focused on individuals with extreme lipoprotein(a) levels and/or corresponding *LPA* risk genotypes, as done previously (6,8) and corresponding to the common sense approach of most doctors. Thus, we conducted analyses only including individuals with lipoprotein(a) levels ≥ 24 th, ≥ 68 th, ≥ 80 th, ≥ 90 th, or > 95 th percentile while ignoring persons with the corresponding lower levels, and similarly for *LPA* risk genotypes. In other words, when examining these different cutpoints for lipoprotein(a) levels and *LPA* genotypes, participants not included were dropped prior to assessment of measures of reclassification and discrimination, while underlying Cox regression models were fitted to data from all participants. For comparison, we also performed analyses including the entire range of lipoprotein(a) levels and *LPA* risk genotypes.

We used Stata version 12.1 (StataCorp LP, College Station, Texas). Two-sided $p < 0.05$ was considered significant. One-way analysis of variance was used to estimate the contribution of *LPA* KIV-2, rs3798220, and rs10455872 genotypes to the variation in plasma lipoprotein(a) levels; lipoprotein(a) levels were, for this analysis solely, square root-transformed due to skewness of the distribution.

Cox proportional hazards regression was used to estimate multivariable adjusted hazard ratios of first-ever MI or CHD events with 95% confidence intervals (CI). We analyzed age at event using left-truncation (delayed entry), and age as time scale. Analyses were multivariable adjusted for age, sex, total cholesterol, HDL cholesterol, systolic blood pressure, smoking, and diabetes mellitus. For this adjustment, total cholesterol values were adjusted for the lipoprotein(a) contribution, as done previously (6,8), and according to compositional data in which cholesterol accounts for ~30% of total lipoprotein(a) mass (21,22). Based on the second lipoprotein(a) measurement in 2001 to 2003, hazard ratios for increased lipoprotein(a) levels were corrected for regression dilution bias (20). We used z tests (23) to test whether hazard ratios for MI differed from hazard ratios for CHD or non-MI CHD.

For estimating measures of reclassification and discrimination, we first constructed a Cox regression model analyzing time to event (stratified by sex) including only conventional risk factors (age, total cholesterol, HDL cholesterol, systolic blood pressure, smoking, and diabetes mellitus). Importantly, for estimating risk prediction measures, cholesterol values were not corrected for the lipoprotein(a) contribution. We here derived the baseline survival at 10 years, $S_0(10)$, for each sex and the beta coefficients, $\beta_1, \beta_2, \dots, \beta_p$, for the included covariates, x_1, x_2, \dots, x_p (Online Tables 1 and 2 list all beta coefficients included in risk prediction models). The probability of surviving beyond 10

years, $P_{\text{surv}}(10)$, after baseline without experiencing an event was calculated as (12)

$$P_{\text{surv}}(10) = S_0(10) \wedge \exp(\beta_1 x_1 + \beta_2 x_2 + \dots + \beta_p x_p)$$

and the probability of an event within 10 years after baseline was 1 minus the survival probability

$$P_{\text{event}}(10) = 1 - P_{\text{surv}}(10)$$

Deaths from causes other than CHD were considered censored events, thus, the previous probability refers to risk of an MI/CHD event in the absence of death from other causes.

Second, we constructed a similar Cox proportional hazards regression model but now including conventional risk factors plus a new risk factor (lipoprotein(a) level on a continuous scale, *LPA* KIV-2 genotype on a continuous scale, *LPA* rs3798220 genotype, and/or *LPA* rs10455872 genotype). Importantly, to avoid favoring lipoprotein(a) in risk prediction, hazard ratios for increased lipoprotein(a) levels were not corrected for regression dilution bias in these analyses. We calculated the new probability of surviving beyond 10 years, $P_{\text{surv_new}}(10)$, without event, as described previously.

The Cox regression models were fitted to data from all participants and then predictive ability was assessed using measures of reclassification and discrimination, as described below. We did not employ cross-validation approaches (i.e., perform model derivation and validation in separate subsets of participants) as the dataset used was of substantial size minimizing the risk of overfitting. According to Phillips et al. (24), the beta coefficients are attenuated to $k\beta$ in a validation sample where $k = 1 - (q - 2)/\chi^2$, q is the number of parameters in the model, and χ^2 is the likelihood ratio chi-square statistic for the model. In our modeling k values exceeded 0.99 in all main analyses indicating overall negligible bias in regression coefficients from overfitting.

We assessed reclassification in participants with extreme concentration/genotype levels and with known event status

at 10 years (i.e., excluding participants censored for other reasons before 10 years), under the assumption that models derived from data on all participants, as described previously, were still applicable. Using the estimated 10-year risk predictions $P_{\text{surv}}(10)$ and $P_{\text{surv_new}}(10)$, participants were classified into 3 a priori defined standard 10-year risk categories of <10%, 10% to 19.9%, and $\geq 20\%$. Cross tabulation of the risk categories was stratified by event (MI or CHD) status at 10 years, and reclassification of participants between risk categories using $P_{\text{surv}}(10)$ versus using $P_{\text{surv_new}}(10)$ was examined. Reclassification was deemed appropriate for participants with events moving up in risk category and for participants without events moving down in risk category on addition of the new risk factor. Reclassification was summarized using the net reclassification index (NRI) and the integrated discrimination improvement (or index) (IDI) (14).

Furthermore, we assessed discrimination over the entire concentration/genotype levels by computing the C index (i.e., Harrell's C statistic) and difference in C indices between models with and without new risk factors (25–27). The C index is the probability that, for a randomly selected pair of participants, the individual with the shorter survival time has the higher value of the predictor ($\beta_1 x_1 + \beta_2 x_2 + \dots + \beta_p x_p$). In our case, the predictor included conventional risk factors or conventional risk factors plus new risk factors, as described previously.

Results

Baseline characteristics of the 8,720 participants are shown in Table 1. We observed 730 and 1,683 first-time MI and CHD events during 17 years, and of these, 453 and 1,012 occurred during the first 10 years of follow-up.

Risk of MI and CHD. For lipoprotein(a) levels ≥ 80 th (≥ 47 mg/dl) or >95 th (>115 mg/dl) percentile, multivariable adjusted hazard ratios were 2.0 (95% CI: 1.5 to 2.7) and 2.5 (1.7 to 3.9) for MI, and 1.5 (1.3 to 1.8) and 1.7 (1.3 to

Table 1 Baseline Characteristics of Participants

	All	Lipoprotein(a) Percentiles					
		<24th	≥ 24 th	≥ 68 th	≥ 80 th	≥ 90 th	>95th
No. of individuals	8,720	2,055	6,665	2,851	1,831	959	436
Women, %	57	53	58	59	60	62	64
Age, yrs	59 (46–69)	57 (44–68)	60 (47–70)	60 (49–70)	60 (49–70)	61 (50–70)	62 (53–71)
Total cholesterol, mmol/l	6.1 (5.2–6.9)	5.8 (5.0–6.7)	6.1 (5.3–7.0)	6.3 (5.4–7.2)	6.4 (5.5–7.3)	6.6 (5.7–7.4)	6.8 (6.0–7.7)
HDL cholesterol, mmol/l	1.5 (1.2–1.9)	1.5 (1.2–1.9)	1.5 (1.2–1.9)	1.5 (1.2–1.9)	1.5 (1.2–1.9)	1.5 (1.2–1.9)	1.6 (1.3–1.9)
Systolic BP, mm Hg	136 (122–152)	135 (121–151)	136 (122–152)	137 (123–153)	138 (124–154)	140 (125–155)	140 (126–156)
Smoking, %	49	51	49	49	49	49	49
Diabetes, %	4	5	4	4	4	4	3
Lipoprotein (a), mg/dl	17 (5–39)	2 (0–3)	25 (13–52)	59 (40–94)	79 (61–113)	112 (93–137)	139 (125–161)
<i>LPA</i> KIV-2, no. of repeats	36 (30–41)	38 (33–43)	35 (29–40)	31 (26–36)	29 (25–34)	28 (24–32)	27 (22–31)
<i>LPA</i> rs3798220, % carriers	2	1	3	5	8	14	24
<i>LPA</i> rs10455872, % carriers	13	1	17	39	52	54	47

Values are n or median (interquartile range).

BP = blood pressure; HDL = high density lipoprotein; KIV-2 = kringle IV type 2.

2.3) for CHD, as compared to participants with levels <24th percentile (Fig. 1).

The *LPA* KIV-2 repeat genotype explained 21% of the total variation in plasma lipoprotein(a) levels and correlated inversely with levels; median lipoprotein(a) levels were 10 mg/dl (interquartile range: 3 to 21 mg/dl), 21 mg/dl (7 to 49 mg/dl), 38 mg/dl (11 to 77 mg/dl), 49 mg/dl (17 to 91 mg/dl), 59 mg/dl (28 to 104 mg/dl), and 70 mg/dl (40 to 123 mg/dl) for number of KIV-2 repeats >77th, ≤77th, ≤33rd, ≤21st, ≤11th, and ≤6th percen-

tiles, respectively. For KIV-2 repeats ≤21st or ≤6th percentiles, multivariable adjusted hazard ratios were 1.5 (95% CI: 1.2 to 1.8) and 1.9 (95% CI: 1.4 to 2.7) for MI, and 1.2 (95% CI: 1.0 to 1.3) and 1.5 (95% CI: 1.2 to 1.8) for CHD, as compared to participants with number of repeats >77th percentile (Fig. 1).

The *LPA* rs3798220 genotype (2% carriers) explained 5% of the total variation in plasma lipoprotein(a) levels. Median lipoprotein(a) levels were 17 mg/dl (interquartile range: 5 to 38 mg/dl) and 116 mg/dl (21 to 158 mg/dl)

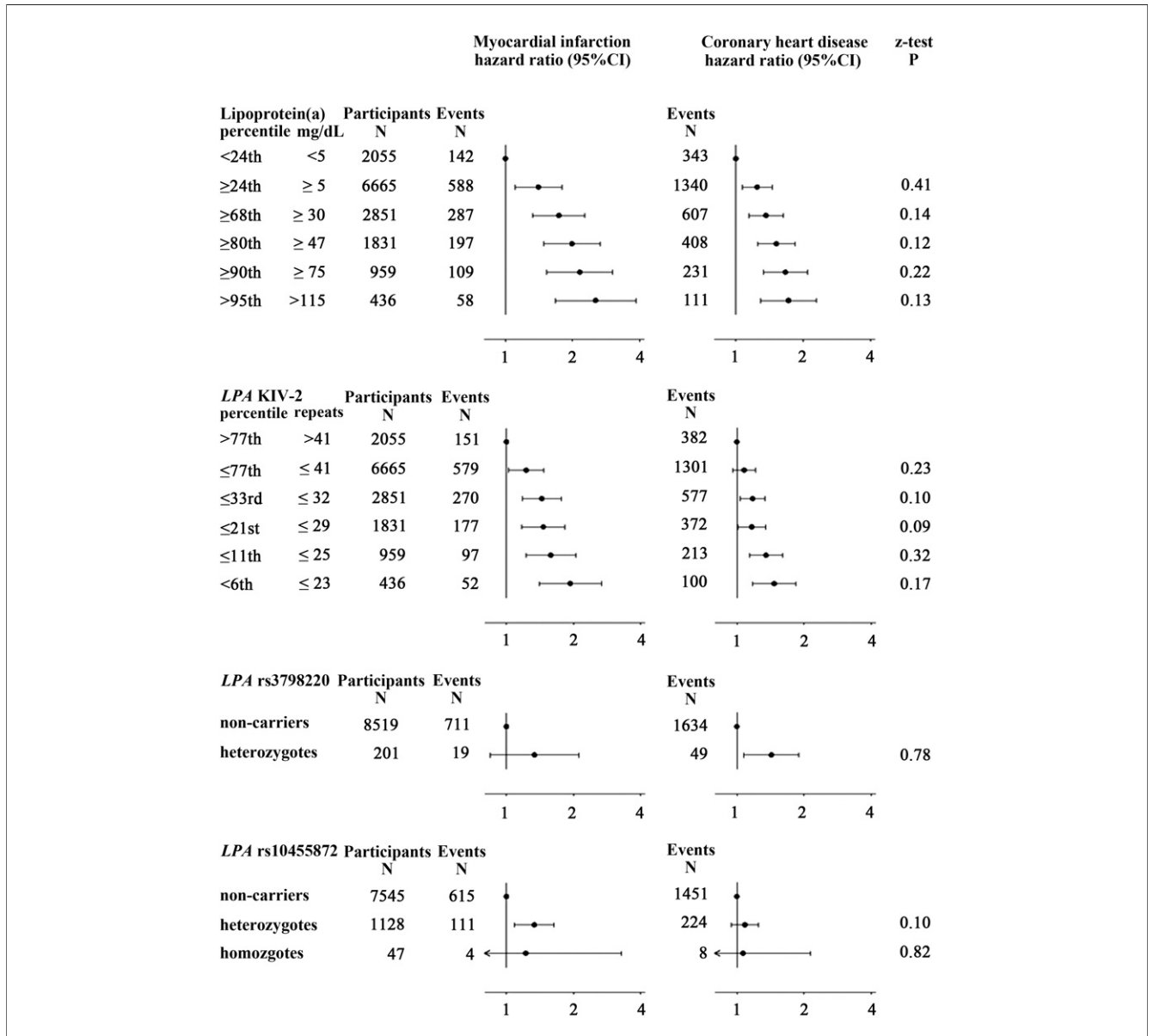


Figure 1 Risk of Myocardial Infarction and Coronary Heart Disease as a Function of Elevated Lipoprotein(a) Levels and Corresponding *LPA* KIV-2, rs3798220, and rs10455872 Risk Genotypes

Based on 8,720 Danish general population participants, the Copenhagen City Heart Study followed from 1991 to 1994 through May 2011 without losses to follow-up. Analyses are multivariable adjusted for age, sex, total cholesterol (corrected for the lipoprotein(a) contribution), high-density lipoprotein cholesterol, systolic blood pressure, smoking, and diabetes mellitus, and also adjusted for regression dilution bias. p Values from z tests show whether there are significant differences between hazard ratios for myocardial infarction and coronary heart disease for each subgroup defined by lipoprotein(a) levels or *LPA* genotype. CI = confidence interval; KIV-2 = kringle IV type 2.

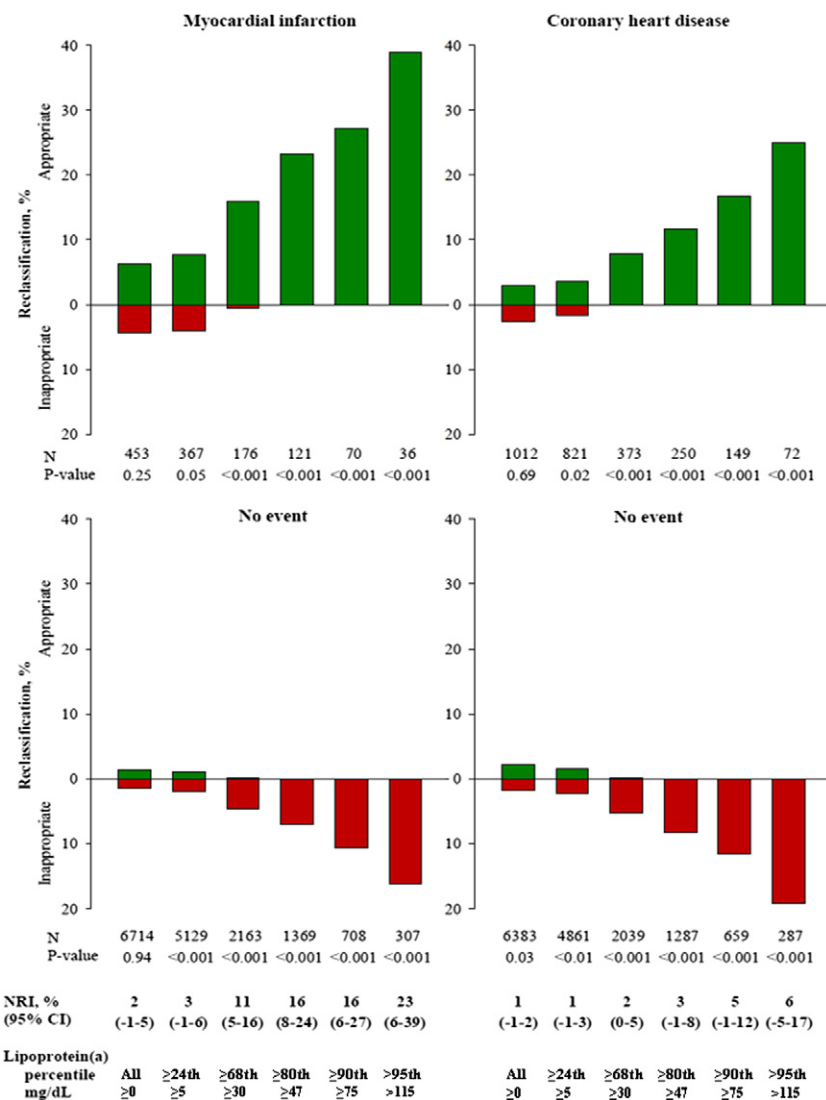


Figure 2 Reclassification as a Function of Elevated Lipoprotein(a) Levels

Based on 8,720 Danish general population participants, the Copenhagen City Heart Study followed from 1991 to 1994 through May 2011 without losses to follow-up. CI = confidence interval; NRI = net reclassification index.

for noncarriers and heterozygotes. Heterozygotes had multivariable adjusted hazard ratios of 1.3 (95% CI: 0.8 to 2.1) for MI, and 1.4 (1.1 to 1.9) for CHD, as compared to noncarriers (Fig. 1).

The *LPA* rs10455872 genotype (13% carriers) explained 27% of the total variation in plasma lipoprotein(a) levels. Median lipoprotein(a) levels were 13 mg/dl (interquartile range: 4 to 28 mg/dl), 69 mg/dl (52 to 98 mg/dl), and 122 mg/dl (103 to 157 mg/dl) for noncarriers, heterozygotes, and homozygotes, respectively. Heterozygotes and homozygotes had multifactorially adjusted hazard ratios of 1.3 (95% CI: 1.1 to 1.6) and 1.2 (95% CI: 0.5 to 3.3) for MI, and 1.1 (95% CI: 0.9 to 1.3) and 1.1 (95% CI: 0.5 to 2.1) for CHD, as compared to noncarriers (Fig. 1).

Comparing MI and CHD hazard ratios yielded z test p values of 0.12 to 0.41 for elevated lipoprotein(a) levels, and of 0.09 to 0.82 for *LPA* risk genotypes (Fig. 1). When comparing MI and non-MI CHD (i.e., excluding individuals who developed MI during follow-up) hazard ratios, z test p values ranged from 0.03 to 0.24 for elevated lipoprotein(a) levels and from 0.01 to 0.68 for *LPA* risk genotypes (Online Fig. 2).

Reclassification and discrimination upon addition of lipoprotein(a) levels. Addition of extreme lipoprotein(a) levels to models based on conventional risk factors improved event classification (Fig. 2), for example, for lipoprotein(a) levels ≥ 80 th (≥ 47 mg/dl) or >95 th (>115 mg/dl) percentiles, 23% ($p < 0.001$) and 39% ($p < 0.001$) of MI events,

and 12% ($p < 0.001$) and 25% ($p < 0.001$) of CHD events, were reclassified correctly, while no events were reclassified incorrectly for either endpoint. For these cutpoints, incorrect reclassification of no events were 7% ($p < 0.001$) and 16% ($p < 0.001$) for MI, yielding NRIs of +16% (8% to 24%) and +23% (6% to 39%), and 8% ($p < 0.001$) and 19% ($p < 0.001$) for CHD, yielding NRIs of +3% (–1% to 8%) and +6% (–5% to 17%) (Fig. 2).

Discrimination was also improved, using these cutpoints, as indicated by IDIs of 0.017 (0.012 to 0.022) and 0.024 (0.011 to 0.037) for MI, and of 0.015 (0.012 to 0.018) and 0.022 (0.015 to 0.029) for CHD.

In comparison, addition of lipoprotein(a) over the entire concentration range to models based on conventional risk factors did not significantly improve reclassification yielding NRIs of +2% (–1% to 5%) and +1% (–1% to 2%) for MI and CHD, respectively. Discrimination was improved slightly, however, as indicated by corresponding IDIs of 0.002 (0.000 to 0.004) and 0.002 (0.001 to 0.003), while C index changes of 0.0024 (–0.001 to 0.0049) and 0.0010 (–0.001 to 0.0021) remained insignificant.

When considering only individuals at 10% to 19.9% absolute 10-year risk (intermediate risk) of MI or CHD based on conventional risk factors, addition of extreme lipoprotein(a) levels to models resulted in an overall more pronounced reclassification of events and non-events (Online Fig. 3). Also, addition of lipoprotein(a) over the entire concentration range, among those at intermediate risk, improved reclassification for MI and CHD yielding NRIs of +7 (0% to 13%) and +6% (3% to 9%).

Reclassification and discrimination upon addition of *LPA* risk genotypes. Addition of information on carrier status for *LPA* risk genotypes to models based on conventional risk factors improved event classification (Table 2, Fig. 3). Results for MI and CHD event and nonevent reclassification for KIV-2 risk genotypes were similar, although slightly attenuated (Fig. 3), compared to those obtained for extreme lipoprotein(a) levels (Fig. 2). NRIs for KIV-2 repeats ≤ 21 st and < 6 th percentile were +12% (5% to 19%) and +12% (–2% to 26%) for MI and +4% (0% to 8%) and +3% (–5% to 11%) for CHD (Fig. 3). Corresponding IDIs for these cutpoints were 0.017 (0.013 to 0.022) (Table 2) and 0.022 (0.013 to 0.032) for MI, and 0.009 (0.008 to 0.011) (Table 2) and 0.013 (0.009 to 0.016) for CHD.

NRIs for *LPA* SNPs rs3798220 and rs10455872 risk genotypes (i.e., minor allele carriers) were +15% (–14% to 44%) and +16% (6% to 26%) for MI, and +10% (–10% to 30%) and +2% (–1% to 6%) for CHD (Table 2). Corresponding IDIs were 0.011 (0.005 to 0.018) and 0.011 (0.008 to 0.015) for MI, and 0.029 (0.018 to 0.040) and 0.002 (0.002 to 0.003) for CHD (Table 2).

In comparison, addition of *LPA* genotypes overall (i.e., including also carriers of *LPA* nonrisk genotypes) to models based on conventional risk factors did not or only very slightly improve reclassification yielding NRIs for MI and CHD of +1% (–3% to 4%) and 0% (–1% to 2%) for

KIV-2 repeats, and of 0% (0% to 1%) and 0% (0% to 1%) for SNP rs3798220, and of +1% (–1% to 3%) and +1% (0% to 1%) for SNP rs10455872. Corresponding IDIs were 0.003 (0.001 to 0.005) and 0.001 (0.000 to 0.002) for KIV-2 repeats, and equaled zero for either endpoint for both *LPA* SNPs. Corresponding C-index changes were 0.0032 (0.0005 to 0.0059) and 0.0007 (–0.0002 to 0.0016) for KIV-2 repeats, and remained insignificant for either endpoint for both SNPs.

When considering only individuals at 10% to 19.9% absolute 10-year risk (intermediate risk) of MI or CHD based on conventional risk factors, addition of *LPA* KIV-2 risk genotypes to models resulted in an overall more pronounced reclassification of events and non-events (Online Fig. 4). Also, addition of *LPA* KIV-2 genotypes overall, among those at intermediate risk, improved reclassification for MI and CHD yielding NRIs of +6% (–2% to 13%) and +7% (4% to 10%).

Reclassification and discrimination upon addition of extreme lipoprotein(a) levels and *LPA* risk genotypes combined. Combining extreme lipoprotein(a) levels (≥ 80 th percentile) with *LPA* KIV-2 and/or SNP risk genotypes did somewhat improve values for NRI and IDI for MI beyond what could be achieved with addition of only extreme lipoprotein(a) levels or corresponding risk genotypes separately (Table 2). This was attenuated for CHD.

Discussion

We here demonstrate that extreme lipoprotein(a) levels or corresponding *LPA* risk genotypes can substantially improve MI and CHD risk prediction in a general population setting, a novel finding (2). Importantly, our common sense approach of focusing exclusively on extreme lipoprotein(a) levels and corresponding *LPA* risk genotypes completely eliminated incorrect reclassification of MI/CHD events, that is, no individuals who developed an event during 10 years of follow-up were incorrectly reclassified down to a lower risk category. Simply put, an approach of widespread lipoprotein(a) measurements (or *LPA* genotyping) to identify individuals with extreme levels may substantially improve MI/CHD risk prediction for this group representing up to one fifth of the general adult population. Alternatively, and to limit cost, lipoprotein(a) screening may be limited to individuals at intermediate risk of CHD (based on conventional risk factors), where reclassification due to extreme lipoprotein(a) levels was even more pronounced. Of note, lipoprotein(a) measurements are also likely to be cost-effective since a single measure of lipoprotein(a) is generally sufficient given the stability of levels over time.

Extreme lipoprotein(a) levels, *LPA* risk genotypes, and risk prediction. A unique feature of lipoprotein(a), as compared to other lipid cardiovascular risk factors such as total, LDL, and HDL cholesterol, is the highly skewed concentration distribution with a long tail toward extremely high values (15). Lipoprotein(a) levels may thus vary up to

Table 2 Values of NRI and IDI for Myocardial Infarction and Coronary Heart Disease for Extreme Lipoprotein(a) Levels, for Risk LPA Genotypes, and for Combinations of Extreme Levels and Risk Genotypes

	Lp(a) ≥80th %	LPA KIV-2 Repeats ≤21st %	LPA rs3798220 Carrier (= Heterozygote)	LPA rs10455872 Carrier (= Heterozygote or Homozygote)	Lp(a) ≥80th % and LPA KIV-2 Repeats ≤21st %	Lp(a) ≥80th % and LPA rs3798220 or rs10455872 Carrier	Combination of All
Myocardial infarction							
NRI, %	16 (8 to 24)	12 (5 to 19)	15 (-14 to 44)	16 (6 to 26)	21 (9 to 32)	19 (8 to 29)	19 (5 to 34)
Events	121	110	9	69	65	65	41
app.	28	20	2	14	21	16	12
inapp.	0	0	0	0	0	0	0
p value	<0.001	<0.001	0.16	<0.001	<0.001	<0.001	<0.001
No event	1,369	1,406	160	925	710	846	494
app.	0	0	0	0	0	0	0
inapp.	96	86	11	40	83	50	50
p value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IDI	0.017 (0.012 to 0.022)	0.017 (0.013 to 0.022)	0.011 (0.005 to 0.018)	0.011 (0.008 to 0.015)	0.025 (0.016 to 0.033)	0.018 (0.010 to 0.025)	0.019 (0.009 to 0.029)
Coronary heart disease							
NRI, %	3 (-1 to 8)	4 (0 to 8)	10 (-10 to 30)	2 (-1 to 6)	4 (-3 to 11)	3 (-2 to 9)	4 (-3 to 12)
Events	250	227	22	142	130	134	83
app.	29	22	6	6	19	13	11
inapp.	0	0	0	0	0	1	0
p value	<0.001	<0.001	0.01	0.01	<0.001	0.001	<0.001
No event	1,287	1,329	152	872	667	795	463
app.	0	0	0	0	0	2	0
inapp.	106	71	26	16	71	47	41
p value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
IDI	0.015 (0.012 to 0.018)	0.009 (0.008 to 0.011)	0.029 (0.018 to 0.040)	0.002 (0.002 to 0.003)	0.016 (0.012 to 0.021)	0.011 (0.005 to 0.017)	0.010 (0.003 to 0.018)

Values are n, unless otherwise stated. 95% confidence intervals for NRI and IDI values are shown in parentheses. Calculations on NRI and IDI include only participants with known cardiovascular disease status at 10-year follow-up. app. = appropriate reclassification; IDI = integrated discrimination index; inapp. = inappropriate reclassification; Lp(a) = lipoprotein(a); NRI = net reclassification index;

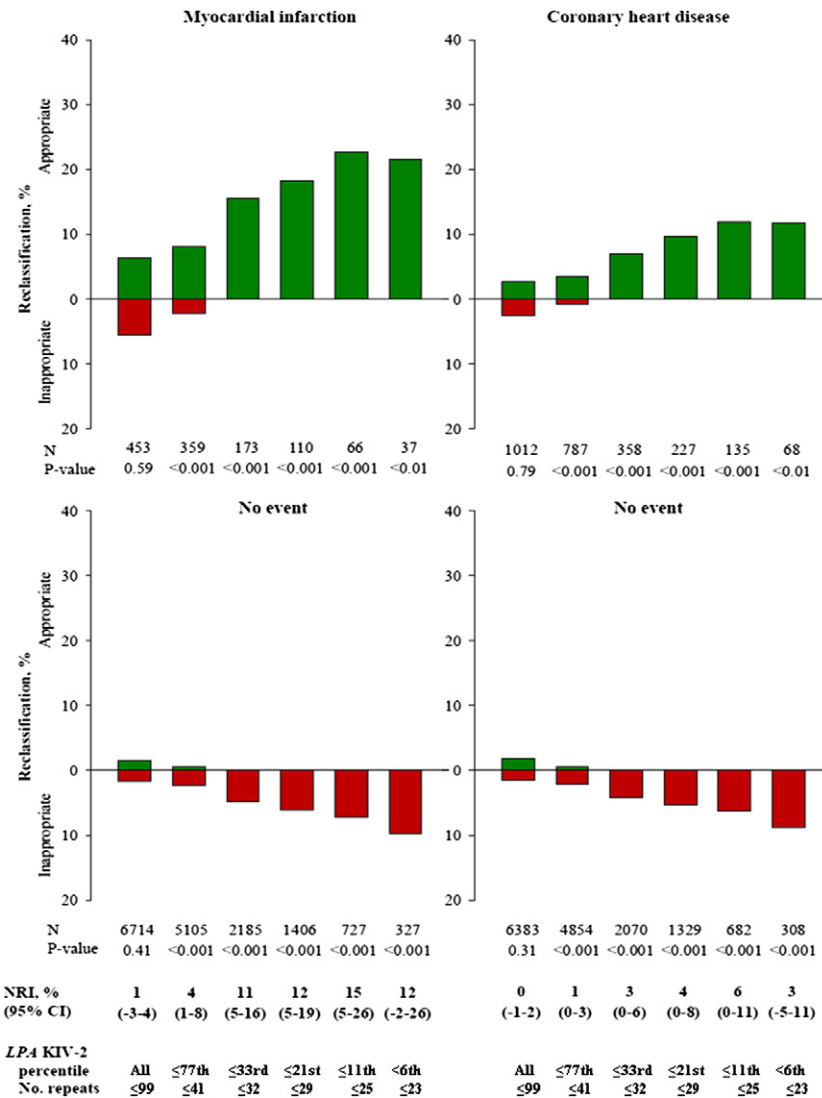


Figure 3 Reclassification as a Function of *LPA* KIV-2 Repeat Genotype

Based on 8,720 Danish general population participants, the Copenhagen City Heart Study followed from 1991 to 1994 through May 2011 without losses to follow-up. Abbreviations as in Figures 1 and 2.

a 1,000-fold among healthy individuals (<0.2 to >200 mg/dl) (15) and approximately 20% of individuals are found in the tail part of the distribution (2). Previous studies have demonstrated that although risk of CHD may increase already at moderately elevated lipoprotein(a) levels, risk is most pronounced for extreme levels (5,6). Thus, considering the entire range of lipoprotein(a) levels in estimating measures of reclassification and discrimination may not adequately capture the potential of extreme levels to substantially improve MI and CHD risk prediction for individuals in the tail part of the lipoprotein(a) distribution. This notion is supported by our results, demonstrating slight or no significant increases in NRIs or improvement of discrimination when considering the entire range of lipoprotein(a)

levels/genotypes, in accordance with recent results from The Emerging Risk Factors Collaboration (12).

Some previous studies have indicated that small apolipoprotein(a) isoforms may be particularly harmful to an extent not fully explained by the associated higher lipoprotein(a) levels (7,17,18). Thus, *LPA* genotypes influencing not only levels of lipoprotein(a), but also reflecting size wise and structural apolipoprotein(a) differences, may potentially add incremental information to lipoprotein(a) levels in CHD risk prediction. We observed similar improvements in measures of reclassification and discrimination for extreme lipoprotein(a) levels and corresponding *LPA* KIV-2 and rs10455872 risk genotypes, while the *LPA* rs3798220 risk genotype did not significantly improve reclassification and

only slightly discrimination. Of note, the rarity of rs3798220 carrier status (2%) severely limits statistical power to detect any predictive effect. When adding both extreme lipoprotein(a) levels and corresponding *LPA* KIV-2 and/or SNP risk genotypes to conventional risk factors, reclassification and discrimination improved somewhat for MI beyond what could be seen for adding solely extreme lipoprotein(a) levels or corresponding *LPA* KIV-2 or rs10455872 risk genotypes. This additive genotype effect may reflect that genetic variants are better markers of long-term lipoprotein(a) levels than single plasma measurements, and/or that small apolipoprotein(a) isoforms (encoded by *LPA* risk genotypes) are particularly harmful and confer atherogenicity beyond effects on hepatic lipoprotein(a) secretion and plasma levels. Although this topic needs further studies and scrutiny, our data do to some extent support adding both extreme lipoprotein(a) levels and corresponding *LPA* risk genotypes to conventional risk factors in risk prediction.

Overall, extreme lipoprotein(a) levels and corresponding *LPA* risk genotypes appeared better predictors of MI than of CHD, and NRI values appeared more pronounced for MI than for CHD. This may reflect a prothrombotic effect of lipoprotein(a), in addition to a proatherosclerotic and/or prostenotic effect, promoting MI in particular. Of note, we have previously reported an increased risk of deep venous thrombosis for (exclusively) extreme lipoprotein(a) levels >95th percentile and corresponding *LPA* KIV-2 risk genotypes (16). Alternatively, the difference may reflect some degree of misclassification of non-MI CHD, a less hard endpoint than MI.

Study limitations. The present study is not the first study to report on lipoprotein(a) and CHD risk prediction (12). However, no previous studies have explored the use of extreme lipoprotein(a) levels and corresponding *LPA* risk genotypes in risk prediction. A limitation of our study is that we only studied white individuals, and thus, results may not necessarily apply to other ethnicities, particularly as lipoprotein(a) levels and distributions differ between different ethnic groups (15). Another limitation of our study is that our KIV-2 genotype reflects the sum of repeats on both alleles, and therefore assumes an additive effect of both alleles on lipoprotein(a) levels. Thus, it is possible that a more refined measure of the KIV-2 genotype may be able to add further incremental value to lipoprotein(a) levels in MI and CHD risk prediction. Finally, we compared MI and CHD hazard ratios using z tests, not entirely appropriate as the 2 endpoints are not independent, however, we also compared MI to non-MI CHD hazard ratios where no diseased participants overlapped.

Conclusions

We provide novel evidence that adding information on extreme lipoprotein(a) levels (or corresponding *LPA* KIV2 or rs10455872 risk genotypes) to conventional risk factors

may substantially improve MI and CHD risk prediction. Additional identified individuals at increased risk may hopefully benefit from aggressive prophylactic measures, including statins for LDL-cholesterol lowering and possibly niacin treatment for lipoprotein(a) lowering, as recently recommended (2).

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Key Words: coronary heart disease ■ lipoprotein(a) ■ risk prediction.

 **APPENDIX**

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

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