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Apolipoprotein(a) Isoforms and the Risk of Vascular Disease

Systematic Review of 40 Studies Involving 58,000 Participants

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Objectives The purpose of this study was to assess the association of apolipoprotein(a) (apo[a]) isoforms with cardiovascular disease risk.

Background Although circulating lipoprotein(a) (Lp[a]) is likely to be a causal risk factor in coronary heart disease (CHD), the magnitude of this association is modest. Lipoprotein(a) particles with smaller, rather than larger, apo(a) isoforms may be stronger risk factors.

Methods Information was collated from 40 studies published between January 1970 and June 2009 that reported on associations between apo(a) isoforms and risk of CHD or ischemic stroke (involving a total of 11,396 patients and 46,938 controls).

Results Thirty-six studies used broadly comparable phenotyping and analytic methods to assess apo(a) isoform size. These studies yielded a combined relative risk for CHD of 2.08 (95% confidence intervals [CI]: 1.67 to 2.58) for individuals with smaller versus larger apo(a) isoforms (corresponding approximately to 22 or fewer kringle IV type 2 repeats vs. >22 repeats or analogously an apo[a] molecular weight of <640 kDa vs. ≥640 kDa). There was substantial heterogeneity among these studies (I^2 = 85%, 80% to 89%), which was mainly explained by differences in the laboratory methods and analytic approaches used. In the 6 studies of ischemic stroke that used comparable phenotypic methods, the combined relative risk was 2.14 (1.85 to 2.97). Overall, however, only 3 studies made allowances for Lp(a) concentration.

Conclusions People with smaller apo(a) isoforms have an approximately 2-fold higher risk of CHD or ischemic stroke than those with larger proteins. Further studies are needed to determine whether the impact of smaller apo(a) isoforms is independent from Lp(a) concentration and other risk factors. (J Am Coll Cardiol 2010;55:2160–7)

Lipoprotein(a) (Lp[a]) is composed of a low-density lipoprotein (LDL) particle and a glycoprotein molecule known as apolipoprotein(a) (apo[a]) (1). Apolipoprotein(a) is structurally homologous to plasminogen and is responsible for the unique properties of Lp(a) (1,2). A collaborative analysis of individual data from 36 prospective studies, involving more than 126,000 individuals, has demonstrated that circulating Lp(a) concentration is continuously associated with risk of coronary heart disease (CHD) and stroke independent from several conventional risk factors (including total cholesterol) (3). The likelihood that Lp(a) is causally relevant to vascular disease risk has been increased by reports of highly significant associations of Lp(a)-related genetic variants with CHD risk (4–9). However, because the risk with Lp(a) concentration is only about one-quarter

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of that seen with LDL cholesterol (3), any clinical implications of this moderate association currently appear limited. Such considerations could change if specific Lp(a) subtypes were shown to confer importantly higher vascular risks. In particular, it has been proposed that Lp(a) particles with smaller apo(a) isoforms may be more pathogenic because they appear to have: 1) increased capacity to bind oxidized phospholipids; 2) greater propensity to localize in blood vessel walls through increased lysine-binding ability and interaction with fibrin; and 3) greater thrombogenic effect through increased inhibition of plasmin activity (10–13). It has also been suggested that smaller apo(a) isoforms may act synergistically with other factors such as small-dense LDL and oxidized LDL particles (10,13–15). The basis for apo(a) size heterogeneity relates to a copy-number variation in one of its protein domains, kringle IV type 2 (KIV_2), which exists in 5 to 50 identically repeated copies. This copy-number variation confers marked heterogeneity in the molecular mass of apo(a) isoforms, which can range between 200 and 800 kDa (Table 1) (1,16,17). Apolipoprotein(a) is encoded by the *LPA* gene, which contains a 5.6-kb segment existing in multiple repeats (KIV_2 repeat polymorphism) that is responsible for the apo(a) isoform variation (2,18).

Many studies (19–23) have reported on the association of apo(a) isoform size variations with the risk of vascular disease. Although they have reported apparently divergent relative risks (RRs), these studies have tended to be small and to involve wide confidence intervals. Their interpretation has been complicated by differences in relation to: 1) populations studied (e.g., people of European, Asian, or African ancestry) because apo(a) characteristics tend to vary by ethnicity (24); 2) methods used to measure apo(a) isoforms (e.g., genotypic vs. phenotypic methods, and among the latter, quantitative vs. semiquantitative approaches); 3) vascular disease outcomes recorded (e.g., myocardial infarction [MI], coronary stenosis, stroke); and 4) analytic approaches used (e.g., different cut-offs chosen to define smaller apo[a] size). Studies have also differed in adjustments for covariates, particularly in relation to circulating Lp(a) concentration, higher levels of which tend to be associated with smaller apo(a) isoforms (4,25,26).

Table 1 Relationship Between Various Approaches Used to Express apo(a) Isoform Sizes

Apo(a) Isoform Size Expressed as		
No. of KIV_2 Repeats	Gel Migration Speed	Molecular Weight
11–13	F	<400 kDa
14–16	B	460 kDa
17–19	S1	520 kDa
20–22	S2	580 kDa
23–25	S3	640–655 kDa
>25	S4	>700 kDa

For gel migration speed, F = mobility faster than apolipoprotein-B_{100} ($apoB_{100}$), B = mobility equal to $apoB_{100}$, and S1–S4 = different levels of mobility slower than $apoB_{100}$. Relevant references are provided in the Online Appendix.

apo(a) = apolipoprotein(a); KIV_2 = kringle IV type 2.

To help clarify the evidence, we have conducted a systematic review and meta-analysis of 40 relevant studies of apo(a) isoforms and coronary and ischemic stroke outcomes that involved a total of 11,396 cases and 46,938 controls.

Abbreviations and Acronyms

apo(a) = apolipoprotein(a)
CHD = coronary heart disease
KIV_2 = kringle IV type 2
LDL = low-density lipoprotein
Lp(a) = lipoprotein(a)
MI = myocardial infarction
RR = relative risk

Methods

Study selection. Studies published between January 1970 and June 2009 that reported on associations between apo(a) isoforms and coronary or stroke outcomes were identified by systematic searches of MEDLINE, scanning of the reference lists of original reports, and discussions with investigators. Electronic searches used MeSH terms and free text related to vascular disease and apo(a) isoforms (e.g., "cardiovascular" [MeSH], "lipoprotein(a)" [MeSH], "protein isoforms" [MeSH], "apolipoprotein(a)," "isoforms," "coronary heart disease," and "stroke"). Studies were eligible for inclusion if they: 1) were broadly population based (i.e., did not select participants or controls on the basis of preexisting comorbidities or cardiovascular risk factors (such as end-stage renal disease, diabetes, or high LDL cholesterol levels); 2) had used a well-described assay to measure apo(a) isoforms; 3) recorded CHD (defined as MI, angina, coronary stenosis, or revascularization) or ischemic stroke outcomes using accepted criteria (i.e., MI using World Health Organization or similar criteria, coronary stenosis using quantitative angiography and typically defined as at least 1 coronary artery with ≥50% coronary stenosis, or ischemic stroke using brain imaging); and 4) provided findings that could be used to calculate an odds ratio for vascular disease. Retrospective and cross-sectional study designs were eligible for inclusion because apo(a) isoforms are determined by copy-number variation in the *LPA* gene (1,2) and are therefore unlikely to be altered by prevalent vascular disease. In cases of apparent duplicate publication, investigators were contacted to confirm whether such studies contained unique participants (lack of reply led to use of the report with the greatest number of participants). Forty unique studies were included (Fig. 1).

Data extraction. The following information was extracted from each article using a standardized abstraction form: study population (including population source and the sampling method employed), geographic location, year of baseline survey, age range of participants at baseline, percentage of male participants, mean duration of follow-up (for prospective studies), vascular disease outcome definition, assay methods and standards used, type of blood sample used, mean apo(a) isoform size and Lp(a) concentration, RR estimates for CHD or ischemic stroke, cut-off level used to categorize apo(a) isoforms as smaller or larger, and degree of statistical adjustment for any potential confounders used (+ = no adjustment; ++ = adjustment for age, sex, and

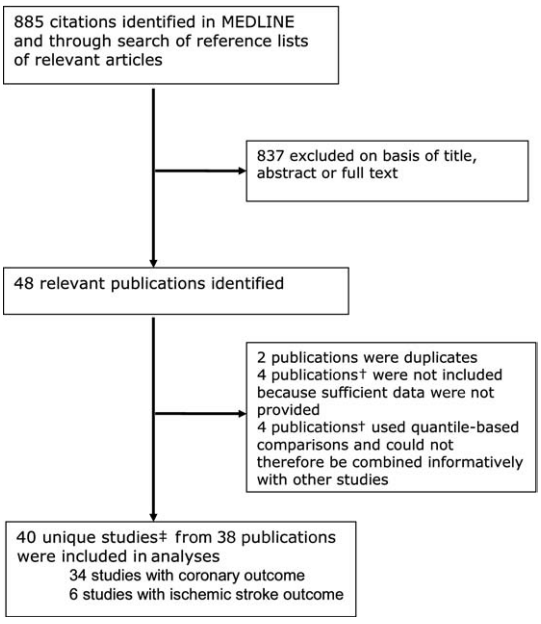

Figure 1 **Study Flow Diagram**

†These studies reported a total of 1,838 CHD cases, approximately 15% of the total included in the current review. ‡The number of studies exceeds the number of articles because 1 publication presented data from 3 studies.

some standard vascular risk factors; +++ = adjustment for the preceding plus Lp[a] concentration).

Statistical analysis. Relative risks for vascular disease were calculated by comparing individuals with smaller-sized apo(a) isoforms with those with larger isoforms. Cut-off levels to define smaller versus larger isoforms were taken as reported in each contributing study. Apolipoprotein(a) isoforms have been reported to have a bimodal distribution in European populations, with a trough in the distribution around 22 KIV_2 repeats (approximately 40% of the general white population has fewer than 22 repeats) (24). This value has been used as the cut-off in most studies that used quantitative electrophoretic approaches to measure apo(a) isoform size (although some studies have used different cut-offs [e.g., 25 or 27 KIV_2 repeats]). Studies that used semiquantitative approaches generally involved comparable cut-off values. In the studies that used electrophoretic methods, RRs were estimated assuming a dominant effect of the risk phenotype (i.e., by comparing people who expressed at least 1 small apo[a] isoform with individuals having 2 large apo[a] isoforms or those who did not express apo[a]). Four studies that used genotypic (i.e., quantitative polymerase chain reaction [PCR] or pulsed-field gel electrophoresis) methods were analyzed separately because they measured the sum of KIV_2 repeats on both alleles, which involves assumptions about additivity of the effects of KIV_2 repeats (see the Discussion section).

When RRs were not reported in publications, they were calculated based on the numbers of cases and controls falling into categories of smaller or larger apo(a) isoforms using the Fisher exact method. Summary RRs for CHD or ischemic stroke were calculated by pooling study-specific estimates using a random-effects meta-analysis (parallel analyses involved fixed-effect models). All analyses were performed using only within-study comparisons to limit possible biases. Consistency of findings across studies was assessed by standard chi-square tests and the I^2 statistic (27). Sources of heterogeneity were investigated by comparing results from studies grouped according to pre-specified study-level characteristics using meta-regression. Evidence of publication bias was assessed using funnel plots and the Egger test (28) and by comparing pooled results from studies involving at least 500 CHD cases with pooled results from smaller studies. All analyses were performed using Stata release 10 (StataCorp, College Station, Texas). Statistical tests were 2-sided and used a significance threshold of $p < 0.05$.

Results

A total of 40 relevant studies (9,12,14,19,21,22,25,29–59) reporting on 58,334 individuals were identified (Table 2). Twenty-seven studies were based in Europe, 5 in East Asia, 2 in the U.S., 3 in South Asia, and 2 in the Middle East; 1 study was multinational (with centers in Austria, Germany, Israel, Wales, China, and India). Overall, 57% of the participants were male, and the weighted mean age at baseline was 56 ± 10 years. Thirty-six studies used electrophoresis to characterize apo(a) isoform size. Of these studies, 15 compared apo(a) gel migration speed against that of apolipoprotein-B_{100}, 17 measured the number of KIV_2 repeats (9 dichotomized the isoforms at 22 KIV_2 repeats, whereas the remainder used cut-off values of 20, 25, 26, or 27 repeats), and 4 studies measured the molecular weight of apo(a). Table 1 summarizes the approximate relationships between these measures. A further 4 studies used genotyping methods, characterizing apo(a) isoforms as total number of KIV_2 repeats.

Thirty studies (12,14,19,21,22,25,29–51,53) that used broadly comparable phenotyping and analytic methods assessed CHD (7,382 cases and 8,514 controls). Using a random-effects model, the combined RR for CHD was 2.08 (95% confidence intervals [CI]: 1.67 to 2.58) in a comparison of individuals with smaller versus larger apo(a) isoforms; the corresponding RR in a fixed-effect model was 1.88 (95% CI: 1.74 to 2.04) (Fig. 2). Only 3 of these studies, however, reported RRs adjusted for Lp(a) concentration. In these studies (463 CHD cases and 298 controls), the combined RR was reduced from 2.26 (95% CI: 1.13 to 4.54) to 1.48 (95% CI: 0.97 to 2.26) after such adjustment. There was evidence of substantial heterogeneity among the 30 studies contributing to the CHD total (I^2 = 85%, 80% to 89%). A considerable portion of this heterogeneity was accounted for by recorded study characteristics, notably differences in definitions used for smaller versus larger apo(a) isoforms (which explained 53% of the observed between-study variation; $p < 0.001$) and type of assay method used ($p = 0.04$) (Fig. 3). There was limited power

Table 2 Summary of 40 Epidemiologic Studies That Assessed the Association Between apo(a) Isoforms and the Risk of CVD

First Author, Year (Ref. #)	Country	Male (%)	Age (yrs)	No. of Cases	No. of Controls	Case Definition	Blood Sample	apo(a) Method*	Comparison† (Cut Point)
Studies of CHD That Used Phenotyping Methods									
Kraft, 1996 (25)	Austria	80	51	69	69	MI, CAD	Plasma	SDS-PAGE§	KIV_2 repeat (20)
Klausen, 1997‡ (30)	Denmark	100	ns	74	190	MI, AP	Plasma	SDS-PAGE	Migration speed (S2)
Emanuele, 2004 (35)	Italy	65	65	83	94	MI, AP	Plasma	SDS-agarose	KIV_2 repeat (25)
Parlavecchia, 1994 (31)	Italy	100	<55	83	96	MI, CAD	Plasma	SDS-PAGE	Migration speed (S2)
Martin, 2002 (19)	Spain	100	<50	91	99	MI	Plasma	SDS-agarose	KIV_2 repeat (25)
Simo, 2001 (12)	Spain	100	<50	95	95	MI	Plasma	SDS-agarose§	KIV_2 repeat (22)
Geethanjali, 2002 (51)	India	ns	53	104	104	CAD	Plasma	SDS-agarose	Migration speed (S2)
Qin, 1995 (47)	China	ns	ns	105	102	MI, CAD	ns	SDS-PAGE	Migration speed (S2)
Zeljkovic, 2009 (14)	Serbia	61	56	109	102	CAD	Plasma	SDS-agarose	KIV_2 repeat (22)
Calmarza, 2004 (32)	Spain	100	<60	111	99	MI	Serum	SDS-PAGE	Migration speed (S2)
Akanji, 2000 (33)	Kuwait	73	55	128	140	MI, CABG	Serum	SDS-PAGE	Migration speed (S2)
Katsouras, 2001 (34)	Greece	72	61	131	33	MI, AP	Plasma	SDS-agarose	KIV_2 repeat (26)
Gazzaruso, 1997 (29)	Italy	83	60	142	264	MI, CAD, AP CABG	Plasma	SDS-agarose	Molecular weight (640 kDa)
Sandholzer, 1991 (48)	Singapore	80	58	162	210	CAD	Plasma	SDS-PAGE	Migration speed (S2)
Sandholzer, 1991 (48)	Singapore	80	58	193	189	CAD	Plasma	SDS-PAGE	Migration speed (S2)
Rifai, 2004‡ (36)	U.S.	100	40–84	195	195	AP	Plasma	SDS-agarose	KIV_2 repeat (22)
Emanuele, 2004 (37)	Italy	84	55	210	105	MI, UAP	Plasma	SDS-agarose	KIV_2 repeat (25)
Gambhir, 2008 (53)	India	87	<40	220	160	CAD	Plasma	SDS-agarose	KIV_2 repeat (22)
Zorio, 2006 (38)	Spain	89	<51	222	199	MI	Plasma	SDS-agarose	Migration speed (S2)
Kalina, 2001 (39)	Hungary	ns	ns	263	97	CAD	ns	SDS-agarose	KIV_2 repeat (22)
Bigot, 1997 (40)	France	84	38–88	267	259	CABG	Serum	SDS-PAGE	Migration speed (S2)
Paultre, 2000 (41)	U.S.	61	56	289	283	CAD	Serum	SDS-agarose§	KIV_2 repeat (22)
Gazzaruso, 1999 (42)	Italy	88	52	335	370	MI, CAD, AP, CABG	Plasma	SDS-Agarose	Molecular weight (640 kDa)
Emanuele, 2003 (45)	Italy	76	62	337	103	MI, CAD, AP, CABG	Plasma	SDS-Agarose	Molecular weight (640 kDa)
Kark, 1993 (44)	Israel	44	54	365	397	MI	Plasma	SDS-PAGE	Migration speed (S2)
Abe, 1992 (49,50)	Japan	86	50	470	465	CAD	Serum	SDS-PAGE	Migration speed (S2)
Brazier, 1999 (21)	Ireland, France	100	25–64	481	519	MI	ns	SDS-agarose	KIV_2 repeat (27)
Holmer, 2003 (22)	Germany	62	51	834	1548	MI	Serum	SDS-PAGE	KIV_2 repeat (22)
Sandholzer, 1992 (46)	Multicenter	86	50–59	1013	1570	CAD	Plasma	SDS-agarose	Migration speed (S2)
Gazzaruso, 2001 (42)	Italy	52	59	201	358	CAD	Plasma	SDS-agarose	Molecular weight (640 kDa)
Studies of CHD That Used Genotyping Methods									
Geethanjali, 2003 (52)	India	70	52	480	254	CAD	Plasma	PFGE	KIV_2 repeat sum (55)
Kamstrup, 2009‡ (9)	Denmark	39	55	599	8038	MI	Serum	QPCR	KIV_2 repeat sum (41)
Kamstrup, 2009 (9)	Denmark	39	59	986	22,265	MI	Serum	QPCR	KIV_2 repeat sum (41)
Kamstrup, 2009 (9)	Denmark	39	60	1231	1230	MI	Serum	QPCR	KIV_2 repeat sum (41)
Studies of Ischemic Stroke									
Yingdong, 1999 (54)	China	50	67	42	85	Ischemic stroke	Serum	SDS-PAGE	Migration speed (S2)
Kronenberg, 1999‡ (55)	Italy	ns	ns	64	826	CVD	Plasma	SDS-agarose	KIV_2 repeat (22)
Peynet, 1999 (56)	France	50	17–54	90	84	Ischemic stroke	Serum	SDS-agarose	KIV_2 repeat (22)
Zambrelli, 2005 (57)	Italy	67	70	94	188	Ischemic stroke	Plasma	SDS-agarose	KIV_2 repeat (26)
Milionis, 2006 (58)	Greece	54	77	163	166	Ischemic stroke	Serum	SDS-agarose	KIV_2 repeat (27)
Jurgens, 1995 (59)	Austria	34	51	265	288	Ischemic stroke or TIA	Serum	SDS-agarose	Migration speed (S2)

*SDS-agarose and SDS-PAGE refer to apo(a) isoform phenotyping techniques using electrophoresis, PFGE is an apo(a) isoform genotyping technique using electrophoresis. †Comparisons were made between individuals with small and large apo(a) isoforms expressed as number of KIV_2 repeats, sum of KIV_2 repeats in both alleles, speed of migration on gel (F, B, S1, S2, S3, S4, 0), and molecular weight in kDa or isoform size quantiles. ‡Prospective studies. §These studies used PFGE to validate apo(a) isoform phenotype measurements.

AP = angina pectoris; CABG = coronary artery bypass graft; CAD = coronary artery disease; CHD = coronary heart disease; CVD = cardiovascular disease; MI = myocardial infarction; na = not applicable; ns = not stated; PFGE = pulsed-field gel electrophoresis; QPCR = quantitative polymerase chain reaction; SDS = sodium dodecyl sulfate; SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis; TIA = transient ischemic attack; UAP = unstable angina pectoris.

to detect differences in many of the subgroups displayed in Figure 3; for example, it was not possible to explore ethnic-related differences because most of the available data related to people of European continental ancestry. Analyses by study size, funnel plots, and Egger test did not reveal evidence for the presence of publication bias (Fig. 3, Online Fig. 1). In the 4 studies (9,52) that used genotypic methods (3,296 cases and 36,787 controls), the combined RR for CHD was 1.19 (95% CI: 1.06 to 1.33) for smaller versus greater number of KIV_2 repeats.

Six studies (55–60) that used broadly comparable electrophoretic methods focused on ischemic stroke (718 cases and 1,637 controls). Using a random-effects model, the combined RR for ischemic stroke was 2.14 (95% CI: 1.85 to 2.97) (Online Fig. 2) in a comparison of individuals with smaller versus larger apo(a) isoforms; the corresponding RR

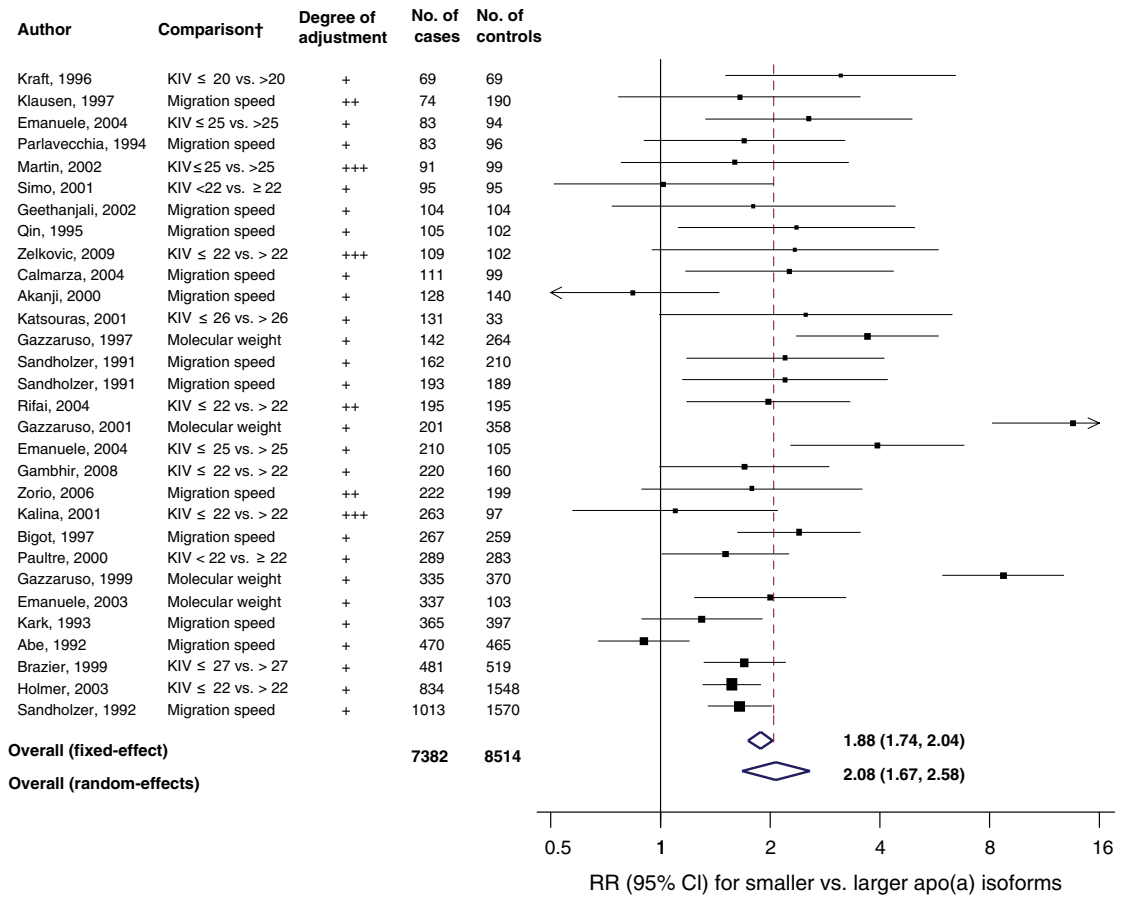

Figure 2 **Apolipoprotein(a) Isoform Size and Risk of CHD Among 30 Studies That Used Comparable Phenotyping Methods and Analytic Approaches**

Forest plot of study-specific associations and overall pooled estimates. Size of data markers is proportional to the inverse of the variance in each study. Assessment of heterogeneity: I^2 = 85% ($p < 0.001$). Fifty-three percent of this variation was explained by the apo(a) isoform size comparison groups ($p < 0.001$). †Migration speed comparisons were between individuals having isoforms with F, B, S1, or S2 gel mobility vs. those having S3 or S4 mobility or null allele; the molecular weight comparisons used a cut-off value of 640 kDa. Degree of adjustment: + = unadjusted; ++ = adjustment for standard risk factors (e.g., age, sex, conventional lipids); +++ = adjustment for preceding plus Lp(a) concentration. CHD = coronary heart disease; CI = confidence interval; RR = relative risk.

in a fixed-effect model was 2.35 (95% CI: 1.86 to 2.97). Again, there was considerable heterogeneity among the studies contributing to this estimate (I^2 = 62%, 8% to 85%). Data on ischemic stroke were too sparse to attempt subgroup analyses.

Discussion

Recent large observational and genetic studies have suggested that Lp(a) concentration is likely to be a causal risk factor in CHD, but the association is comparatively moderate in magnitude (i.e., an RR of approximately 1.3 in a comparison of people in the top one-third with those in the bottom one-third of the population distribution) (3). Consequently, there is interest in whether certain subtypes of Lp(a) may be more strongly associated with disease risk. The current systematic review of 40 studies, involving more than 58,000 participants, indicates that people with smaller apo(a) isoforms have an approximately 2-fold higher risk of CHD (and ischemic stroke) than those with larger proteins. This risk relates approximately to a comparison of people with 22 or fewer KIV_2 repeats versus those with >22 repeats (or analogously, an apo[a] molecular weight of <640 kDa vs. ≥640 kDa). These 2 groups encompass approximately 40% and 60%, respectively, of the general white population (30,56,58). Furthermore, although the current meta-analysis focused on studies of general populations, associations of similar magnitude have been observed for vascular risk with apo(a) isoforms in high-risk populations such as patients with hypertension (60), hypercholesterolemia (35), or diabetes (61). Hence, available data encourage study of apo(a) isoforms in cardiovascular risk prediction and in randomized trials of agents that can lower Lp(a) concentration (e.g., niacin or certain inhibitors of cholesteryl ester transfer protein) (62,63).

An important limitation, however, is the general lack of adjustment in the available data of associations between apo(a) isoforms and CHD for Lp(a) concentration. In people of European continental ancestry, apo(a) isoform

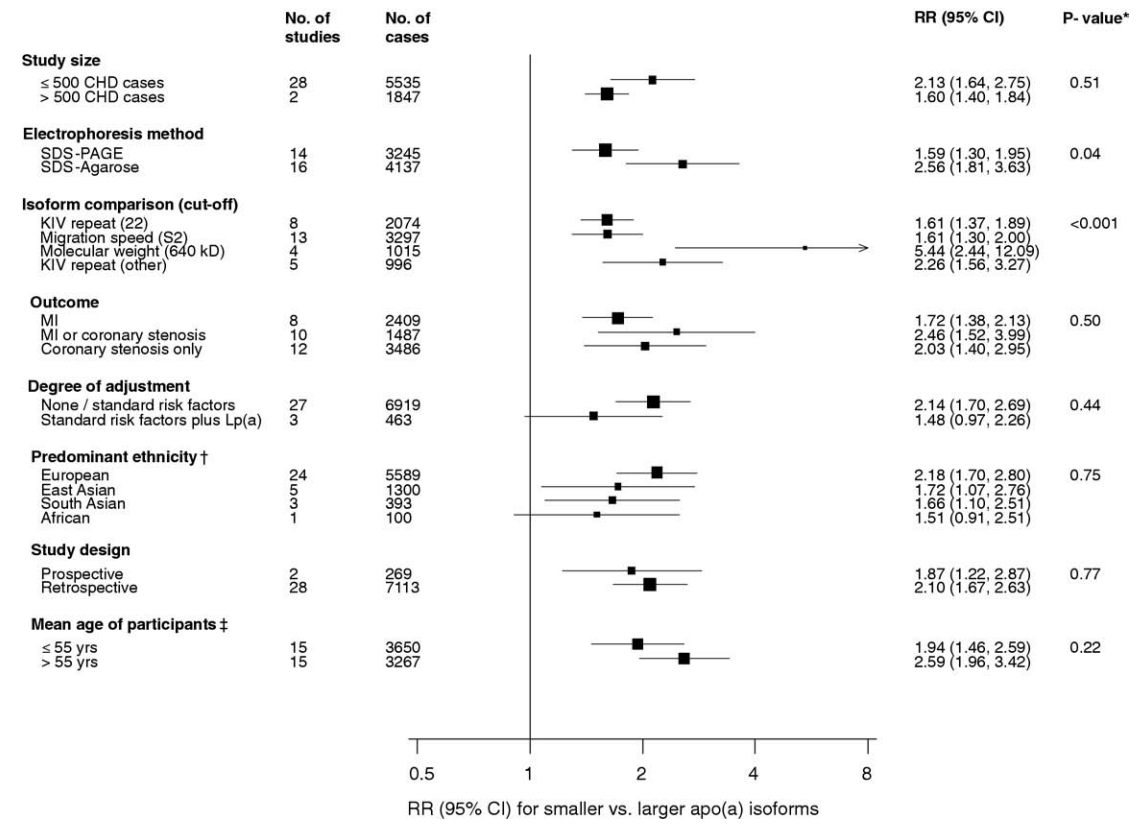

Figure 3 **Apolipoprotein(a) Isoform Size and CHD Risk Grouped by Recorded Study-Level Characteristics**

Pooled associations with CHD risk in relevant subgroups. Size of data markers is proportional to the inverse of the variance in each study. For the ethnicity, sex, and age subgroups, studies may have contributed data to more than 1 category. *p values for heterogeneity from meta-regressions. †Two studies contributed to more than 1 category of ethnicity. ‡Two studies did not provide information on age, and 2 studies provided information on both categories of age. CHD = coronary heart disease; CI = confidence intervals; MI = myocardial infarction; PFGE = pulsed-field gel electrophoresis; RR = relative risk; SDS = sodium dodecyl sulfate; SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis.

polymorphism contributes between 40% and 70% of the variation in Lp(a) concentration, with fewer number of KIV_2 repeats being associated with higher Lp(a) concentration (4,25,26). It is likely, therefore, that at least part of the association observed between apo(a) isoforms and CHD risk in the current review is mediated by Lp(a) concentration. Because only 3 available studies have adjusted associations of apo(a) isoform with CHD for Lp(a) concentration, however, it remains difficult to judge to what extent associations of apo(a) isoforms and vascular disease depend on Lp(a) concentration (55,64). Although it is clear that large-scale studies of CHD are needed, with concomitant assays of apo(a) isoforms and Lp(a) concentration, a potential difficulty is the labor-intensive nature of conventional methods to measure apo(a) isoforms. Furthermore, interpretation of data on apo(a) isoform phenotypes may be complicated by: 1) difficulty in detecting apo(a) isoforms with fewer than 15 KIV_2 repeats (which encompass about 3% of the general white population) (24); 2) potential difficulties in distinguishing heterozygotes with similarly sized isoforms; and 3) potential difficulties in distinguishing between nonexpressed alleles and homozygous phenotypes. One approach to address these limitations is to use supplementary information on KIV_2 repeat polymorphisms in the *LPA* gene, for example, by employing real-time PCR assays (an approach that also facilitates high-throughput measurements) (65). Use of this genotypic approach alone, however, is potentially limited because it measures the sum of KIV_2 repeats in both alleles (rather than the number of repeats in each allele), which implies an additive effect of the number of repeats. This assumption is inconsistent with observations that different KIV_2 repeats are not equally expressed; for example, alleles with fewer than 22 KIV_2 repeats are expressed in more than 90% of individuals, whereas those with >22 repeats are expressed in approximately 50% (with the expression rate decreasing as the number of repeats increases) (23). Hence, this genotypic approach to apo(a) isoform assessment may be liable to misclassify isoform size categories, potentially leading to underestimation of the true associations. Such assay considerations could account for the considerably lower RRs for CHD seen in the current analysis with studies that used real-time PCR compared with those that used conventional electrophoretic methods. More generally, analytic and assay differences between

available studies accounted for much of the heterogeneity noted in the current analysis. Hence, further work is needed to optimize approaches to apo(a) isoform assessment in large studies.

Although the current literature-based meta-analysis has provided the most comprehensive assessment yet of apo(a) isoforms and risk of vascular disease, it has relied on aggregated published data. As such, it was not possible to adjust uniformly for potential confounding factors nor investigate vascular medication usage. Large new studies are, therefore, needed to evaluate potentially important features of this risk relationship, such as the shape of any dose-response curve and most importantly, the extent of independence of apo(a) isoforms from Lp(a) concentration. It is not possible to discount completely the influence of selective reporting on the current review, despite the lack of strong evidence for publication bias. For example, it may be that in some studies, cut-off levels for apo(a) isoform size were chosen only after exploration of the data. Although apo(a) isoforms are determined by copy-number variation in the *LPA* gene (and hence not likely to be affected by cardiovascular disease status), the retrospective design of many of the studies included in this review could be a source of other types of biases, such as selection bias. Evaluation of apo(a) isoforms in prospective studies in the future will provide more robust data. As Lp(a) concentrations tend to vary considerably across different ethnic groups (41,66), further studies are needed in nonwhite populations. In addition, there is a need for detailed phenotyping of participants to help assess potential joint effects of apo(a) isoforms with circulating levels of small-dense LDL and oxidized phospholipids (10,13–15).

Conclusions

People with smaller apo(a) isoforms have an approximately 2-fold higher risk of CHD or ischemic stroke than those with larger proteins. Further studies are needed to determine whether smaller apo(a) isoforms are relevant to vascular disease independent from Lp(a) concentration and other risk factors.

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Key Words: lipoprotein(a) ■ apolipoprotein(a) isoforms ■ cardiovascular disease ■ meta-analysis ■ epidemiology.

APPENDIX

For supplementary figures and the relevant references for Table 1, please see the online version of this article.