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Meta analysis of candidate gene variants outside the *LPA* locus with Lp(a) plasma levels in 14,500 participants of six White European cohorts

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Abstract

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Background—Both genome-wide association studies and candidate gene studies have reported that the major determinant of plasma levels of the Lipoprotein (a) [Lp(a)] reside within the *LPA* locus on chromosome 6. We have used data from the Human CVD bead chip to explore the contribution of other candidate genes determining Lp(a) levels.

Methods—48,032 single nucleotide polymorphisms (SNPs) from the Illumina Human CVD bead chip were genotyped in 5,059 participants of the Whitehall II study (WHII) of randomly ascertained healthy men and women. SNPs showing association with Lp(a) levels of $p < 10^{-4}$ outside the *LPA* locus were selected for replication in a total of an additional 9,463 participants of five European based studies (EAS, EPIC-Norfolk, NPHSII, PROCARDIS, and SAPHIR)

Results—In Whitehall II, apart from the *LPA* locus (where p values for several SNPs were $< 10^{-30}$) there was significant association at four loci *GALNT2*, *FABP1*, *PPARGC1A* and *TNFRSF11A*. However, a meta-analysis of the six studies did not confirm any of these findings.

Conclusion—Results from this meta analysis of 14,522 participants revealed no candidate genes from the Human CVD bead chip outside the *LPA* locus to have an effect on Lp(a) levels. Further studies with genome-wide and denser SNP coverage are required to confirm or refute this finding.

Keywords

Lipoprotein(a); LPA; Illumina Human CVD bead chip; genetic association

Introduction

Lipoprotein(a) or Lp(a) is a lipoprotein particle consisting of an LDL-cholesterol particle with an additional single molecule of the protein apolipoprotein (a) [apo(a)] which is linked by a di-sulphide bond to apolipoprotein B. It is produced in the liver and circulates in the plasma (1; 2). Levels vary widely between individuals, and in many people levels are barely detectable by conventional assays, while in some levels are as high as 100 mg/dl (3). Lp(a) is recognised as an independent risk factor for atherosclerotic cardiovascular disease (4; 5), although the precise pathogenetic mechanism is still unclear.

The Lp(a) protein is encoded by the *LPA* gene, which arose as a duplication of the plasminogen gene in the primate lineage. Plasminogen is a zymogen involved in the coagulation and thrombolysis cascade (6), but mutations in the *LPA* gene have destroyed enzyme function. Plasma levels of Lp(a) are highly heritable, with an estimated heritability of 85% (7), and currently there are no other plasma protein with heritability estimates far above 75%. Most of this genetic determination is due to variation at the *LPA* locus itself (8–10). Though a number of variants in the promoter and elsewhere in the gene contribute, the major determinant is variation in the number of “Kringle-IV” repeats found in the mature protein due to stably inherited duplication of the exon encoding this repeat, with longer proteins being found in subjects with lower levels (11). This has been confirmed by both genome-wide association studies (GWAS) and candidate gene studies. A recent GWAS of plasma Lp(a) concentration (12) suggested a complex genetic architecture of Lp(a) that might involve multiple loci on chromosome 6. A second study the Human CVD bead chip, similar to our study, confirmed the impact of the *LPA* locus and especially the Kringle IV copy number variants (13), and the strong effect of these variants on risk of Coronary Heart Disease (CHD). Both the GWAS and SNP studies involved small sample sizes of less than 900 individuals.

The aim of the current study is to explore the potential role of genetic variants outside the *LPA* locus in influencing plasma levels of Lp(a) in a large population of White Europeans. While the majority of the genome variability involved in determining plasma levels of Lp(a) is likely to be at the *LPA* locus, identifying additional loci may give novel insight into the

control process for Lp(a) production or clearance and may indicate novel therapeutic approaches to lower Lp(a) plasma levels.

Methods

In the discovery stage, analysis was carried out using 48,032 SNPs from the Illumina human CVD bead chip (14) in 5,059 individuals from the Whitehall II (WHII) study (15). The resulting Manhattan plot is provided in Figure S1, with details of genotyping and quality control in Supplementary Methods and (16). The top ranking SNPs from outside the *LPA* locus were selected to be included in the second stage, the meta-analysis. The significance level was set to $p < 2.0 \times 10^{-4}$ for at least one SNP per locus. The strength of statistical evidence does not meet other published thresholds, for example (17; 18), but we selected the most significant SNPs outside the *LPA* gene with the hypothesis that some of these may represent true associations with Lp(a) levels. Additional analysis conditioning on the most significant SNP from *LPA* is in Supplementary Results. Characteristics of the participants are in Table 1 and Supplementary Methods. Lp(a) was natural log transformed to produce a distribution closer to normal, and SNP associations were tested by linear regression using an additive genetic model adjusted for age and sex using PLINK (19). The top ranked SNPs from the discovery analysis were examined with five additional cohorts: Northwick Park Heart Study II [NPHSII] (20), the Edinburgh Artery Study [EAS] (21), the Salzburg Atherosclerosis Prevention Program in subjects at High Individual Risk study [SAPHIR] (22; 23); the Precocious Coronary Artery Disease Study [PROCARDIS] (24) and the European Prospective Investigation into Cancer and Nutrition study- Norfolk [EPIC-N] (25). Characteristics of these cohorts are in Table 1, with further details in Supplementary Methods. Analyses were performed separately in each cohort and were combined using R (www.r-project.org) applying both fixed and random effects models. Results from the random effects model are presented in Table 2, and from the fixed effects model are in Supplementary Table S4.

Results

At the *LPA* locus, the most strongly associated SNP was rs10455872 ($p = 3.45 \times 10^{-283}$) at the discovery stage. This SNP has been reported to be strongly associated with Lp(a) and CHD(26). We identified six SNPs from four loci other than *LPA* to be associated with plasma Lp(a) levels. This was based on a top ranked SNP per gene in addition to a proxy SNP if available. rs2296065 mapped to *GALNT2* on chromosome 1 with p -value = 5.9×10^{-5} , two SNPs mapped to *PPARGC1A* on chromosome 4 (rs2932971 and rs2932976 with p values = 4.4×10^{-4} and 1.9×10^{-4} , respectively), two SNPs from *TNFRSF11A* on chromosome 18 (rs7231887 and rs17069904 with p -values = 4.15×10^{-5} and 1.58×10^{-3} , respectively) and one SNP from *FABP1* on chromosome 2 (rs2919872, $p = 8.98 \times 10^{-5}$). There was no association between these six SNPs outside the *LPA* locus and any other lipid trait or CVD risk factor at $p < 0.01$ (Table S2). The *LPA* SNP rs10455872 explained 24% of the variance of Lp(a) in the WHII study.

The top *LPA* SNP and the six other SNPs from the other four loci were selected for genotyping in an additional 5,253 subjects from three other studies: NPHSII, EAS and SAPHIR. Two other studies were also included in the meta-analysis: PROCARDIS with an extra 1,822 samples by using genotypes from the human CVD bead chip and EPIC-N with 2,388 samples by using genotypes from Affymetrix 500 and imputed data using IMPUTE (27). Mean levels varied between the different cohorts as shown in Table 1, with the highest levels of Lp(a) observed in the PROCARDIS data, where individuals were ascertained on cardiovascular disease. Results from the meta-analysis of the six studies are presented in Table 2. There was an association with $p = 2.15 \times 10^{-3}$ with a small and consistent effect

only between one SNP on *TNFRSF11A* (rs17069904) shown in Table 2. The major contributor to the significance of this effect was the WHII study, which contributed 35% to the total data. In a meta-analysis of the five “replication” studies (excluding WHII), p-values from all SNPs (except rs10455872) were non-significant ($p > 0.05$) as shown in Table 3.

When the analysis in WHII was repeated conditional on the *LPA* SNP rs10455872, eight additional SNPs in six additional genes crossed our significance threshold of $p < 10^{-5}$ (Supplementary Table S3). However none of these SNPs were replicated in the PROCARDIS data set (Table S3) and therefore were not investigated further.

Discussion

We have used the Human CVD bead chip to explore the contribution of genes outside the *LPA* locus to the variation of Lp(a) plasma levels. We initially conducted an association analysis between 48,000 SNPs from the human CVD bead chip and Lp(a) levels measured in 5,059 men and women from the WHII study. Results from this showed significant associations of Lp(a) with variants in four loci additional to *LPA*: *GALNT2*, *FABP1*, *PPARGC1A* and *TNFRSF11A*. We aimed to replicate these findings with a meta-analysis on a further five studies (EAS, EPIC-Norfolk, NPHS2, SAPHIR and PROCARDIS) where the only remaining association was with variants in *TNFRSF11A*, albeit mainly driven by WHII (Figure 1 and Table S3). *TNFRSF11A* (tumor necrosis factor receptor superfamily, member 11a) also known as *RANK* is a protein coding gene. The protein encoded by this gene is a member of the TNF-receptor superfamily. A relationship between *TNFRSF11*-osteoclast differentiation and lipoproteins has been described in (28). *GALNT2* (Polypeptide N-acetylgalactosaminyltransferase 2) has recently been confirmed in GWAS meta analysis as a locus determining HDL-C and triglyceride levels (29; 30) and its influence on Lp(a) was tested in (29) where they tested the association between index SNPs from 30 loci with Lp(a), and the p-value was 2.8×10^{-1} .

Overall, our results provide little evidence that common variants in the cardiometabolic genes on the Human CVD bead chip, outside those at the *LPA* locus, have a major influence on Lp(a) levels, although we cannot rule out the potential influence of other loci in the genome not covered on the array. A limitation of this study is the lack of data on Kringle IV repeats, which is not available for the Whitehall II cohort, and therefore we are unable to look further into this.

A genome-wide association study (GWAS) on 386 individuals from a Hutterite community (a founder population) reported association with SNPs from nine independent genes on chromosome 6 (31) using the Affymetrix 500K chip. They reported one associated SNP on chromosome 13, 79 Kb downstream of *TRPC4*. Another study (32) used the human CVD bead chip genotyped in a multiethnic sample with approximately 900 individuals from South Asian, Chinese and White European origins. Their study reported a strong association between the *LPA* SNP rs10455872 with Lp(a) levels, and that kringle-4 type 2 (KIV-2) copy number explains an increment in plasma Lp(a) variation over SNPs alone. We have chosen rs10455872 as a proof of principle in our meta-analysis based on results from our discovery data set. This SNP also reported in (33), is only prevalent in White Europeans. Two other studies carried out a genome-wide linkage scan, one in Spanish families ascertained on idiopathic thrombophilia in 387 individuals belonging to 21 families (34) and the other on a population-based cohort of 939 men and 1141 women from Finland (35). Both studies confirmed previous results with a strong linkage signal at the *LPA* gene. The Spanish study also report a new locus on Chromosome 2 (in proximity with a number of genes) involved in the quantitative variation of Lp(a), however they did not replicate this finding. For none of

these reported loci were we able to confirm or repudiate the effects since they were not covered in the human CVD bead chip, and there were no other nearby SNPs.

The heritability of Lp(a) is not fully explained by variation due to the *LPA* locus. Lanktree et al. (36) used the same human CVD bead chip as in our study, however only looked at SNPs within the *LPA* locus, and had a small sample size. Given that the major genetic factor that influence Lp(a) levels accounts for a high percentage of the variance, other genes that have a small effect on Lp(a) would be more difficult to find, because of insufficient statistical power, study design or sample size (37).

Rare and structural variation, and heterogeneity between meta-analysis cohorts in our study in regards to different ascertainment criteria will contribute to lack of replication. Additionally, measurement imprecision could also contribute to the lack of association as it reduces opportunities to detect an association and may be greater for Lp(a) levels determined based on frozen rather than fresh samples (39) and assays sensitive to apolipoprotein(a) isoforms (40). However, because environmental factors have very little effect if any on plasma Lp(a) levels, which are not responsive to lipid lowering drugs except for Niacin (38), it is unlikely that environmental differences between cohorts could explain the lack of replication.

In summary, we report a cardio-chip gene-centric association analysis with Lp(a) levels. Our hypothesis was that genomic regions influencing Lp(a) concentration are not limited to the *LPA* gene on chromosome 6. The suggestive evidence of loci other than *LPA* influencing Lp(a) variation found in the discovery data set was not confirmed after combining data in a meta-analysis comprising five studies. We did not observe markedly significant associations between SNPs and Lp(a) levels which suggests that common variants in and around the *LPA* locus are the major contributors to the heritability of Lp(a). The remainder of the heritability was not explained by variants from this gene centric chip, these variants could be untyped, rare, copy number or structural such as insertion/deletions and inversions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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SAPHIR

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PROCARDIS

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EPIC-Norfolk

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EAS

The EAS has been funded by grants from the British Heart Foundation. We are grateful to all study participants and general practitioners who took part in the study

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LPA- rs1055872 Meta-analysis using a fixed effects model

TNFRSFF11A- rs17069904 Meta-analysis using a fixed effects model **Figure 1.**

Forest plots for the five studies and seven SNPs for rs10455872 (LPA) and rs17069904 (TNFRSFF11A).

Table 1

Summary description of the five studies included in the meta-analysis

Study	n	Ascertainment	Age	Assay Method	Lp(a) mg/dl [*]
WHII	M 3721	Random	48 (44–54)	Immunoturbidimetric	19 (11–39)
	F 1338		49 (44–55)		19 (11–44)
EAS	M 446	Random	64 (60–69)	ELISA	11 (4–31)
	F 457		64 (59–69)		10 (5–29)
NPHS2	M 2758	Random	56 (53–59)	ELISA	9 (3–27)
PROCARDIS	M 1294	CAD	62 (57–67)	Latex-enhanced	31.0 (14–85)
	F 528		63 (58–68)	Immunoturbidimetric assay	42.5 (17–97)
SAPHIR	M 1041	Random	49 (44–53)	ELISA	13.8 (6.5–45.7)
	F 551		56 (52–59)		17.8 (7.9–51)
EPIC- Norfolk (sample)	M 797	Random	59.6 (40–77)	Immunoturbidimetric assay	21.5 (0.6–162.3)
	F 882		58.9(40–77)		22.3(1.6–166.7)
EPIC- Norfolk (Obese- BMI> 30)	M 297	Obesity (BMI > 30)	60.2(39–76)	Immunoturbidimetric assay	9.9 (4.6–20.9)
	F 412		58.9(40–76)		13.3 (5.9–28.9)

* Numbers represent median (inter-quartile range)

Table 2

Meta analysis association results for seven SNPs and 14,522, individuals from the six cohorts using a random effects model.

Chr	Gene	SNP	Allele	MAF*	P	Effect size (SE)	QE p-value**
1	<i>GALNT2</i>	rs2296065	A	0.16	6.31×10^{-2}	-0.04 (0.02)	1.53×10^{-1}
2	<i>FABP1</i>	rs2919872 ***	A	0.43	1.08×10^{-2}	0.04 (0.02)	4.82×10^{-1}
4	<i>PPARGC1A</i>	rs2932971	C	0.24	8.43×10^{-1}	0.01 (0.03)	4.15×10^{-3}
4	<i>PPARGC1A</i>	rs2932976	G	0.24	7.96×10^{-1}	0.01 (0.03)	2.56×10^{-3}
6	<i>LPA</i>	rs10455872	A	0.08	3.06×10^{-116}	1.33 (0.06)	8.76×10^{-7}
18	<i>TNFRSF11A</i>	rs7231887	G	0.10	1.11×10^{-1}	-0.05 (0.03)	7.82×10^{-2}
18	<i>TNFRSF11A</i>	rs17069904	G	0.10	2.15×10^{-3}	0.07 (0.02)	4.93×10^{-1}

* MAF is reported for WHII, MAFs for all cohorts are in Supplementary Table S1.

** P-value from heterogeneity test

*** All SNPs are intronic, rs2919872 is 5 upstream.

Table 3

Meta-analysis for Lp(a) using a random effects model with data from EAS, NPHSII, SAPHIR, PROCARDIS, EPIC-N sample and EPIC-N Obese (excluding WHII).

SNP	P-value	Beta	SE	QE p-value*
rs2296065	0.61	−0.01	0.025	0.66
rs2919872	0.42	0.02	0.019	0.97
rs2932971	0.17	0.03	0.020	0.23
rs2932976	0.13	0.03	0.020	0.30
rs10455872	2.03×10^{-102}	1.30	0.029	0.001
rs7231887	0.54	−0.02	0.028	0.42
rs17069904	0.24	−0.04	0.030	0.63

* P-value from heterogeneity test