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LDL Receptor Polymorphisms and the Risk of Coronary Heart Disease: the Atherosclerosis Risk in Communities Study

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Mutations in the low density lipoprotein receptor (*LDLR*) result in ineffective clearance of serum low density lipoprotein (LDL) cholesterol and contribute to premature atherosclerosis and cardiovascular disease (CVD) in familial hypercholesterolemia [1]. Several mutations of the *LDLR* have been described, affecting exons, splicing sites and the promoter region (summarized in [2]). Mutations in genes of other proteins involved in LDL uptake and metabolism (ApoB and LDL receptor adaptor protein – LDLRAP1) and in LDLR intracellular recycling (proprotein convertase subtilisin/kexin type 9 serine protease, PCSK9) have also been implicated in familial hypercholesterolemia [2]. More recently, *PCSK9* mutations associated with reduced serum LDL cholesterol were associated with a lower risk of coronary heart disease (CHD) in the Atherosclerosis Risk in Communities (ARIC) study [3].

The contribution of common variants in the *LDLR* gene to CHD risk has been recently documented in white individuals but not in African Americans [4–7]. In addition, most reported associations are restricted to prevalent CHD. Single nucleotide polymorphisms (SNPs), particularly those located in a regulatory region of the gene, may subtly affect the atherosclerotic process and/or progression through changes in the clearance of LDL cholesterol or by other unknown mechanisms. In this study, we evaluated the association of *LDLR* genetic polymorphisms with incident CHD and all cause mortality among ARIC participants, and if the association of *LDLR* variants with CHD risk was modified by protective mutations in the *PCSK9* gene.

We studied 14,790 genotyped ARIC participants, a bi-racial prospective study of of subclinical and clinical atherosclerosis (1987–1989) [8]. Study participants were selected as a probability sample from four US communities, examined at baseline and at three triennial follow-up exams (Supplement Table 1, baseline study characteristics). Annual follow-up continues to ascertain vital status as well as CHD and stroke, including hospitalizations and deaths. We excluded 42 individuals that were not white or African American, 539 with prevalent CHD, 937 using lipid lowering medications at baseline visit and 136 with missing covariates. CHD events were defined as acute (definitive or probable) myocardial infarction (MI), fatal CHD, EKG diagnosis

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of MI, and coronary revascularization procedures (coronary angioplasty or coronary artery bypass graft) obtained by surveillance through December 2004.

Two single nucleotide polymorphisms in the 5' region of the 3' UTR regulatory region of *LDLR* [rs1433099 (C/T) and rs2738466 (A/G)] were genotyped using TaqMan assays [9]. Genotyping of the *PCSK9* mutations in ARIC has been previously described [3]. The frequencies of the rs1433099 T allele were 0.54 and 0.27 for African American and white individuals, respectively. For rs2738466, the frequencies of the G allele were 0.17 for African Americans and 0.26 for whites. No deviation from Hardy Weinberg equilibrium were noted in race-stratified analysis ($\alpha=0.01$).

Race-specific incidence rates of CHD and all cause mortality per 1,000 person-years were estimated by dividing the number of events by person-time at risk. In each race stratum, the association of *LDLR* genotypes with CHD events or all cause mortality was estimated using Cox proportional hazard models (hazard ratios, HR, and 95% confidence intervals, CI). Initial models adjusted for age, age², sex, age-by-sex interaction and center (model 1), then hypertension, diabetes, body mass index (BMI), current smoking, baseline LDL and HDL cholesterol (model 2). We assumed a codominant inheritance model (2 df), which makes no assumptions about the underlying genetic model [11].

Mean follow-up time was 15 years, with 380 CHD (11%) events among 3,438 African American, and 1,298 CHD (13%) events among 9,698 white individuals. The overall age and gender adjusted incidence rate of CHD was 6.2/1,000 person-years (95% CI, 5.4 to 7.2/1,000 person-years) for African Americans and 5.1/1,000 person-years (95% CI, 4.5 to 5.7/1,000 person-years) for whites.

The incidence of CHD was increased in African Americans with one or two copies of the T allele of rs1433099 (Table 1). When contrasted to participants not carrying a copy of the T allele, carrying 1 copy of the T allele of *LDLR* rs1433099 was associated with a 23% increased risk of CHD, while carrying 2 copies was associated with a 47% increase in risk of CHD events (model 1). Adjusting for CHD risk factors (model 2) slightly attenuated the association with CHD among African American individuals (HR 1.38, 95% CI 1.02, 1.87, $p=0.02$ for two copies of the T allele of rs1433099). This association was not present in whites.

rs2738466 was not associated with CHD in African Americans (Table 1) and none of the *LDLR* SNP genotypes were associated with CHD among whites (Supplement Table 2). *LDLR* genotypes were also not associated with all cause mortality among African American or white individuals.

After excluding the protective mutations of the *PCSK9* gene (N=80 for African Americans: 57 individuals C679X heterozygous, 22 Y142X heterozygous, and 1 individual heterozygous for both mutations; N=307 for whites for R46L mutation)[3], the T allele of rs1433099 was still associated with increased hazard of CHD in African Americans (HR 1.21, 95% CI 0.92, 1.61 and HR 1.46, 95% CI 1.08, 1.96, for carrying one or two copies, respectively, of the rs1433099 T allele compared with carrying no copies of the T allele, model 1). We did not observe a significant association among genotypes and CHD in white individuals after excluding *PCSK9* mutations, even when adjusting for lipid measures. In addition, no changes were observed with regard to the association of genotypes with mortality.

LDLR polymorphisms have been associated with prevalent CHD among white individuals [4,5,7]. In this study, we showed that a common variant in the 3' regulatory region of the *LDLR* gene is associated with increased incident CHD among African Americans. Our results support a significant genetic effect of two copies of the T allele on CHD, but we also observed a trend of increased risk for individuals with 1 copy of the T allele. The hazards for CHD for

this SNP association were unaffected by adjusting for known CHD risk factors or by excluding individuals with known *PCSK9* protective mutations.

Recent studies have shown that polymorphisms in genes affecting LDL and HDL cholesterol are associated with CVD in white individuals [4,7]. Willer et al. found a significant allelic association among the *LDLR* SNP rs6511720 and CHD in the Wellcome Trust Case Control Consortium (WTCCC) and among an expanded sample of 13,000 British individuals[7]. A recent study of six case-control European samples showed a significant protective effect of the T allele of the *LDLR* rs2228671 variant on CHD, with the genetic effects mediated through changes in LDL cholesterol levels [5].

Increased LDL cholesterol has been associated with incident CHD events among ARIC participants [10]. However, the rs1433099 genotypes were not associated with higher LDL cholesterol among ARIC African Americans [9]. In addition, our estimates were not attenuated by adjustment for baseline LDL and HDL cholesterol. Similar findings were described by Kathiresan et al. for the risk score estimates when adjusted for baseline lipid levels[4], and were attributed to the variability of single lipid measures and to the lack of measures on lifetime exposure.

The *LDLR* has a major role in regulating cholesterol clearance by receptor mediated endocytosis of LDL cholesterol in the hepatocytes, but alternative pathways of LDL cholesterol clearance occur through scavenger receptors [11]. Scavenger receptors on macrophages bind modified forms of LDL cholesterol such as oxidized LDL. Earlier atherosclerosis vascular lesions consist mostly of lipid-laden macrophages [12]. Therefore, increased cholesterol clearance by these alternative pathways may promote endothelial cell damage and atherosclerosis without detectable changes in serum lipids. The role of scavenger pathways in individuals with the T allele of rs1433099 should be further explored [13].

Interesting, in a recent analyses of 70 to 85 year-old white participants of the PROSPER trial, which randomized individuals with documented CVD or at least one CVD risk factor to pravastatin or placebo, the *LDLR* rs1433099 SNP was associated with a reduced rate of CVD events at follow up among men assigned to receive pravastatin (HR 0.73, 95% CI 0.55, 0.97) [6]. In this study, another SNP, rs2738466, was associated with increased CVD events. Differences in definition of CVD events (for example, first incident cases in ARIC and first event after drug intervention in PROSPER), population characteristics (older age and more comorbidities in PROSPER) and exposure to anti-lipid drugs may explain some of these findings. In addition, by inhibiting the cholesterol synthesis, statins increase the expression of the *LDLR* but also its degradation by inducing *PCSK9* synthesis [14]; the net genetic effect may vary by context of genetic and environmental influences. Nevertheless, the role of polymorphisms in the *LDLR* gene in response to drug therapy should be further explored.

We did not observed associations among the *LDLR* polymorphisms and CHD in white individuals. Population-specific linkage disequilibrium, differences in environmental exposures, and genetic heterogeneity may have contributed to these findings.

In summary, polymorphisms in the 3' regulatory region of the *LDLR* gene are associated with CHD among African American individuals recruited from the general US population but not among white individuals in the same cohort. Future studies are needed to replicate our finding in African Americans and to address the lack of generability to white individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Rader DJ, Cohen J, Hobbs HH. Monogenic hypercholesterolemia: new insights in pathogenesis and treatment. *J Clin Invest* 2003;111:1795–1803. [PubMed: 12813012]
2. Soutar AK, Naoumova RP. Mechanisms of disease: genetic causes of familial hypercholesterolemia. *Nat Clin Pract Cardiovasc Med* 2007;4:214–225. [PubMed: 17380167]
3. Cohen JC, Boerwinkle E, Mosley TH Jr, Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N Engl J Med* 2006;354:1264–1272. [PubMed: 16554528]
4. Kathiresan S, Melander O, Anevski D, Guiducci C, Burt NP, Roos C, Hirschhorn JN, Berglund G, Hedblad B, Groop L, Altshuler DM, Newton-Cheh C, Orho-Melander M. Polymorphisms associated with cholesterol and risk of cardiovascular events. *N Engl J Med* 2008;358:1240–1249. [PubMed: 18354102]
5. Linsel-Nitschke P, Gotz A, Erdmann J, Braenne I, Braund P, Hengstenberg C, Stark K, Fischer M, Schreiber S, El Mokhtari NE, Schaefer A, Schrezenmeier J, Rubin D, Hinney A, Reinehr T, Roth C, Ortlepp J, Hanrath P, Hall AS, Mangino M, Lieb W, Lamina C, Heid IM, Doering A, Gieger C, Peters A, Meitinger T, Wichmann HE, König IR, Ziegler A, Kronenberg F, Samani NJ, Schunkert H. Lifelong reduction of LDL-cholesterol related to a common variant in the LDL-receptor gene decreases the risk of coronary artery disease--a Mendelian Randomisation study. *PLoS ONE* 2008;3:e2986. [PubMed: 18714375]
6. Polisecki E, Muallem H, Maeda N, Peter I, Robertson M, McMahon AD, Ford I, Packard C, Shepherd J, Jukema JW, Westendorp RG, de Craen AJ, Buckley BM, Ordovas JM, Schaefer EJ. Genetic variation at the LDL receptor and HMG-CoA reductase gene loci, lipid levels, statin response, and cardiovascular disease incidence in PROSPER. *Atherosclerosis* 2008;200:109–114. [PubMed: 18261733]
7. Willer CJ, Sanna S, Jackson AU, Scuteri A, Bonnycastle LL, Clarke R, Heath SC, Timpson NJ, Najjar SS, Stringham HM, Strait J, Duren WL, Maschio A, Busonero F, Mulas A, Albai G, Swift AJ, Morken MA, Narisu N, Bennett D, Parish S, Shen H, Galan P, Meneton P, Hercberg S, Chen WM, Li Y, Scott LJ, Scheet PA, Sundvall J, Watanabe RM, Nagaraja R, Ebrahim S, Lawlor DA, Ben-Shlomo Y, Davey-Smith G, Shuldiner AR, Collins R, Bergman RN, Uda M, Tuomilehto J, Cao A, Collins FS, Lakatta E, Lathrop GM, Boehnke M, Schlessinger D, Mohlke KL, Abecasis GR. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. *Nat Genet* 2008;40:161–169. [PubMed: 18193043]
8. The Atherosclerosis Risk in Communities (ARIC) Study: design and objectives. The ARIC investigators. *Am J Epidemiol* 1989;129:687–702. [PubMed: 2646917]
9. Muallem H, North KE, Kakoki M, Wojczynski MK, Li X, Grove M, Boerwinkle E, Wilhelmsen KC, Heiss G, Maeda N. Quantitative effects of common genetic variations in the 3'UTR of the human LDL-receptor gene and their associations with plasma lipid levels in the Atherosclerosis Risk in Communities study. *Hum Genet* 2007;121:421–431. [PubMed: 17273844]
10. Sharrett AR, Ballantyne CM, Coady SA, Heiss G, Sorlie PD, Catellier D, Patsch W. Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: The Atherosclerosis Risk in Communities (ARIC) Study. *Circulation* 2001;104:1108–1113. [PubMed: 11535564]
11. Goldstein JL, Ho YK, Basu SK, Brown MS. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. *Proc Natl Acad Sci U S A* 1979;76:333–337. [PubMed: 218198]
12. Gerrity RG, Naito HK. Ultrastructural identification of monocyte-derived foam cells in fatty streak lesions. *Artery* 1980;8:208–214. [PubMed: 7213030]

13. Hachem SB, Mooradian AD. Familial dyslipidaemias: an overview of genetics, pathophysiology and management. *Drugs* 2006;66:1949–1969. [PubMed: 17100406]
14. Tall AR. Protease variants, LDL, and coronary heart disease. *N Engl J Med* 2006;354:1310–1312. [PubMed: 16554535]

Incident rates and hazard ratios of coronary heart disease and all cause mortality among ARIC African American individuals by *LDLR* genotypes

Table 1

		Coronary Heart Disease				All Cause Mortality	
		Population	Events	Incident Rates [*] per 1,000 p-y (95% CI)	Hazard Ratio ^{**} (95% CI)	Incident Rates [*] per 1,000 p-y (95% CI)	Hazard Ratio ^{**} (95% CI)
rs1433099							
CC	722	66	4.1 (2.8, 6.1)	1	11.3 (8.9, 14.4)	1	
CT	1,702	186	6.5 (5.3, 8.0)	1.23 (0.93, 1.62)	12.6 (10.9, 14.6)	1.14 (0.94, 1.38)	
TT	1,014	128	7.2 (5.6, 9.2)	1.47 (1.09, 1.98)	12.0 (9.9, 14.5)	1.08 (0.87, 1.34)	
rs2738466							
AA	2,343	266	7.8 (3.4, 17.4)	1	12.1 (10.7, 13.7)	1	
AG	998	100	5.6 (4.2, 7.5)	0.85 (0.68, 1.07)	12.3 (10.1, 14.9)	1.00 (0.9, 1.2)	
GG	97	14	6.4 (5.4, 7.6)	1.34 (0.78, 2.30)	12.0 (6.3, 22.8)	1.11 (0.7, 1.7)	

CHD, coronary heart disease; p-y, person-years; CI, confidence intervals; analysis assume a codominant inheritance model (2 df)

* for a mean age 54 years, adjusted for sex and center

** adjusted for age, age², sex, age-by-sex interaction and center (model 1).