DNA methylation and HDL functionality: the REGICOR study.

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ABSTRACT

Objective: The function of high-density lipoproteins (HDL) may better reflect their atheroprotective role, compared to HDL-cholesterol levels. The association between DNA methylation and HDL function has not yet been established.

Approach and Results: We designed an epigenome-wide association study including 645 individuals from the REGICOR study. We determined DNA methylation from peripheral blood cells using the HumanMethylation450 array. We analyzed HDL functionality by determining HDL cholesterol efflux capacity (CEC) and HDL inflammatory index (HII). We discovered three methylation sites located in *HOXA3*, *PEX5* and *PER3* related to CEC, and one located in *GABRR1* related to HII. Using a candidate gene approach, we also found two methylation sites located in *CMIP* related to CEC.

Conclusions: We identified six potential loci associated with HDL functionality in *HOXA3*, *PEX5*, *PER3, CMIP* and *GABRR1*. Additional studies are warranted to validate these findings in other populations.

Abbreviations

HDL: High-density lipoprotein.

HDL-c: High-density lipoprotein cholesterol.

CHD: Coronary heart disease.

CEC: Cholesterol efflux capacity.

HII: HDL inflammatory index

CpG: Cytosine-phosphate-guanine.

EWAS: Epigenome-wide association studies.

INTRODUCTION

Low levels of high-density lipoprotein (HDL) cholesterol are associated with an increased risk of coronary heart disease (CHD). However, the causal relationship between HDL-cholesterol concentration and CHD is questioned by clinical trials and Mendelian randomization studies;[1,2] therefore, HDL function may better reflect its protective role. Two of the most important HDL functions, cholesterol efflux capacity (CEC) and HDL inflammatory index (HII), have been associated with the incidence of cardiovascular events and acute coronary syndromes.[3,4] Finally, DNA methylation, the heritable and reversible addition of a methyl group to a cytosine-phosphate-guanine (CpG), is associated with HDL-cholesterol levels,[5] but there is no evidence about its role on HDL functional properties.

Our aim was to assess the association between DNA methylation, CEC and HII using an epigenome-wide association study (EWAS) approach and also exploring candidate genes.

MATERIALS AND METHODS

Available in the online-only Supplement.

RESULTS

Clinical characteristics of the 645 participants are available in Supplementary Table I. The correlation coefficient between the two analyzed HDL functions was -0.736 (p-value=0.010).

EWAS approach

The Manhattan and q-q plots are shown in Supplementary Figures I-IV. We identified 2 CpGs, Cg01964852 (*HOXA3* -homeobox A3-) and Cg22812457 (*PEX5* -peroxisomal biogenesis factor 5-), associated with CEC in a model adjusted for confounders, surrogate variables and HDL-cholesterol levels. We also identified the association

between Cg01964852 (*PER3* -period circadian clock 3-) and CEC in the model unadjusted for HDL-c levels. These three CpGs explained 5.38% of the CEC variability (Table 1). Finally, we found an association between Cg25671376 (*GABRR1* -gamma-aminobutyric acid type A receptor, rho1 subunit-), and HII, explaining 0.83% of CEC variability (Table 1).

We looked at the public results of the CARDIoGRAMplusC4D consortium and identified variants in *PER3*, *HOXA3* and *GABRR1* showing suggestive associations with CHD (p-values= 0.0006, 0.0027 and 0.0089, respectively) (Supplementary Table II).

Candidate gene approach

Using the same individuals and a pre-defined selection of 2004 CpGs located in genes previously related to HDL-cholesterol levels (Supplementary table III),[5-7] we identified Cg08876518 and Cg03212183 (both in *CMIP* –c-Maf-inducing protein) associated with CEC, in the model unadjusted for HDL-cholesterol levels (Table 1). These CpGs explained 3.70% of the CEC variability. No significant associations with HII were found.

Additional analyses

The characteristics of the participants across methylation levels of the six CpGs of interest, the correlation between methylation levels, HDL functionality and classical cardiovascular risk factors are shown in the Supplementary Tables IV-XI. We also analyzed the two-way interactions between the CpGs, and the CpGs and obesity and diabetes on HDL functionality and did not found any significant interaction (Supplementary Tables XII-XIII).

DISCUSSION

We identified six CpGs located in five genes –*HOXA3*, *PER3*, *PEX5*, *CMIP* and *GABRR1*– showing differential methylation associated with HDL functionality: CEC or HII. CEC is the ability of HDLs to remove cholesterol excess from cells and has been

related to hematopoietic stem cell mobilization and extramedullary hematopoiesis suppression, leading to decreased production of monocytes and neutrophils and lower atherosclerosis burden.[8] A small proportion of CEC variability was explained by the methylation of CpGs located in *HOXA3, PER3, PEX5* and *CMIP*. *HOXA3* expression is associated with high abdominal adiposity,[9] *PER3* expression is altered in vascular smooth muscle cells in atherosclerotic plaques,[10] *PEX5* is a key gene in the peroxisome formation that is responsible for several lipid metabolism processes,[11] and a *CMIP* polymorphism is associated with HDL-c levels.[6,7] Methylation in these genes was inversely associated with CEC, independently of HDL-cholesterol levels.

HII is an indicator of HDL function related to the inflammatory process in atherogenic plaques.[4] Hypomethylation of *GABRR1* is associated with lower HII levels (more anti-inflammatory HDLs), although the variability explained is small. To the best of our knowledge, there is no relation between *GABRR1* and lipid metabolism or functionality.

Genetic variants in *PER3*, *HOXA3* and *GABRR1* show potential associations with CHD in the CARDIoGRAMplusC4D consortium, suggesting a causal role of these genes in CHD development that could be mediated by HDL functionality.

Our study has some limitations. First, the cross-sectional design did not allow us to infer causality. Second, we could not replicate the results in an independent cohort; to mitigate this limitation, we explored public genome-wide association data (CARDIoGRAMplusC4D) to identify genetic variants in these loci that could be associated with CHD. Our major strength was the use of standardized protocols, the powerful statistical method, and the adjustment for residual confounding factors.

To summarize, we identified five loci potentially associated with HDL functionality in *HOXA3*, *PEX5*, *PER3, CMIP* and *GABRR1.* This is the first evidence of associations between DNA methylation and HDL function. Further research to validate these associations is warranted.

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Disclosures

None.

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Highlights

- This is the first study to provide evidences of associations between DNA methylation and HDL function.
- Hypomethylation of *HOXA3, PEX5, PER3* and *CMIP* was associated with high cholesterol efflux capacity.
- Hypomethylation of *GABRR1* was associated with high HDL anti-inflammatory capacity.

Table 1: CpGs associated with cholesterol efflux capacity or HDL inflammatory Index.

Epigenome-wide approach							
Cholesterol Efflux Capacity							
CpG	**Gene**	**Model 1**		**Model 2**		**Variability (%)**	**Total variability (%)**
		Coeff(SE)	**Pval**	**Coeff(SE)**	**P-value**		
cg04837231	*PER3*	-0.201(0.046)	$1.38x10^{-05}$	-0.271(0.049)	$4.04x10^{-08}$	2.43	5.38
cg01964852	*HOXA3*	-0.184(0.033)	$2.86x10^{-08}$	-0.223(0.041)	$5.04x10^{-08}$	3.84	
cg22812457	*PEX5*	-0.241(0.043)	$1.82x10^{-08}$	-0.219(0.053)	$3.02x10^{-05}$	1.11	
HDL inflammatory Index							
CpG	**Gene**	**Model 1**		**Model 2**		**Variability (%)**	**Total variability (%)**
		Coeff(SE)	**Pval**	**Coeff(SE)**	**P-value**		
cg25671376	*GABRR1*	0.229(0.043)	$7.42x10^{-08}$	0.228(0.042)	$7.62x10^{-08}$	0.83	0.83
Gene candidate approach							
Cholesterol Efflux Capacity							
CpG	**Gene**	**Model 1**		**Model 2**		**Variability (%)**	**Total variability (%)**
		Coeff(SE)	**Pval**	**Coeff(SE)**	**P-value**		
cg08876518	*CMIP*	-0.130(0.032)	$3.94x10^{-05}$	-0.173(0.036)	$1.82x10^{-06}$	3.03	3.70
cg03212183	*CMIP*	-0.124(0.033)	$1.53x10^{-04}$	-0.165(0.038)	$1.40x10^{-05}$	2.51	

Model 1 adjusted for sex, age, smoking, estimated cell count, surrogate variables and HDL-cholesterol levels.
Model 2 adjusted for sex, age, smoking, estimated cell count and surrogate variables.
Coeff(SE): coefficient and standard error of the standardized M-value.