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The shared allelic architecture of adiponectin levels and coronary artery disease

Zari Dastani [a], Toby Johnson [b,c,d], Florian Kronenberg [e], Christopher P. Nelson [f,g], Themistocles L. Assimes [h], Winfried März [i], CARDIoGRAM Consortium, ADIPOGen Consortium J. Brent Richards [j,k,*]

[a] Department of Epidemiology, Biostatistics and Occupational Health, Jewish General Hospital, Lady Davis Institute, McGill University Montreal, Quebec H3T 1E2, Canada
[b] Department of Medical Genetics, University of Lausanne, 1005 Lausanne, Switzerland
[c] University Institute of Social and Preventative Medicine, Centre Hospitalier Universitaire Vaudois (CHUV) and University of Lausanne, Lausanne, Switzerland
[d] Swiss Institute of Bioinformatics, Lausanne, Switzerland
[e] Division of Genetic Epidemiology, Innsbruck Medical University, 6020 Innsbruck, Austria
[f] Department of Cardiovascular Sciences, University of Leicester, Leicester LE3 9QP, UK
[g] National Institute for Health Research (NIHR), Leicester Cardiovascular Disease Biomedical Research Unit, Glenfield Hospital, Leicester LE3 9QP, UK
[h] Department of Medicine, Stanford University School of Medicine, Stanford, CA, USA
[i] Synlab Center of Laboratory Diagnostics Heidelberg, Eppelheim, Germany
[j] Department of Twin Research and Genetic Epidemiology, King's College London, London SE1 7EH, UK
[k] Departments of Medicine, Human Genetics, Epidemiology and Biostatistics, Lady Davis Institute, Jewish General Hospital, McGill University Montreal, Quebec H3T 1E2, Canada

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ABSTRACT

Objective: A large body of epidemiologic data strongly suggests an association between excess adiposity and coronary artery disease (CAD). Low adiponectin levels, a hormone secreted only from adipocytes, have been associated with an increased risk of CAD in observational studies. However, these associations cannot clarify whether this relationship is causal or due to a shared set of causal factors or even confounding. Genome-wide association studies have identified common variants that influence adiponectin levels, providing valuable tools to examine the genetic relationship between adiponectin and CAD.
Methods: Using 145 genome wide significant SNPs for adiponectin from the ADIPOGen consortium ($n = 49{,}891$), we tested whether adiponectin-decreasing alleles influenced risk of CAD in the CARDIoGRAM consortium ($n = 85{,}274$).
Results: In single-SNP analysis, 5 variants among 145 SNPs were associated with increased risk of CAD after correcting for multiple testing ($P < 4.4 \times 10^{-4}$). Using a multi-SNP genotypic risk score to test whether adiponectin levels and CAD have a shared genetic etiology, we found that adiponectin-decreasing alleles increased risk of CAD ($P = 5.4 \times 10^{-7}$).
Conclusion: These findings demonstrate that adiponectin levels and CAD have a shared allelic architecture and provide rationale to undertake a Mendelian randomization studies to understand if this relationship is causal.

1. Introduction

Coronary artery disease (CAD) is the leading cause of death in developed countries. ENREF_1 According to world health organization, CAD is responsible for approximately half of the 36 million deaths from non-communicable diseases [1]. Epidemiological studies have revealed numerous CAD risk factors such as obesity, dyslipidemia, hypertension, smoking and diabetes mellitus. Among these factors, obesity is one of the most prevalent causes of

* Corresponding author. Departments of Medicine, Human Genetics, Epidemiology and Biostatistics, Lady Davis Institute, Jewish General Hospital, McGill University Montreal, Quebec H3T 1E2, Canada. Tel.: +1 514 340 8222x4362; fax: +1 514 340 7529.
E-mail address: brent.richards@mcgill.ca (J. Brent Richards).

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cardiovascular morbidity and mortality [2]. Excess body fat is associated with dysregulation of several bioactive factors produced from adipose tissue which may participate in the development of obesity-related diseases [3].

Adiponectin, a hormone produced predominantly by adipocytes, has anti-atherogenic and anti-inflammatory properties. Low circulating adiponectin levels have been linked to a range of important clinical parameters including blood glucose, indices of insulin resistance, proatherogenic dyslipidemia, risk of type 2 diabetes (T2D), stroke and CAD1 [4,5]. In a study of 225 male patients with documented CAD, compared with age-matched controls, lower plasma adiponectin levels (<4.0 μg/mL), were associated with a 2-fold higher prevalence of CAD, independent of established CAD risk factors [6]. Also, Dzielińska et al. showed that plasma adiponectin levels were decreased in a group of 99 hypertensive men with CAD, as compared to normotensive healthy subjects [7]. Similarly, a nested case-control study among 18,225 male participants in the Health Professionals Follow-up Study revealed that after adjustment for common CAD risk factors, participants in the highest, compared with the lowest, quintile of circulating adiponectin levels had a significantly lower risk of MI (RR = 0.41; 95% CI: 0.24–0.70, $P < 0.001$) [5]. Lawlor et al. investigated the association of adiponectin with incidence of CAD in 4286 women who were randomly selected from 23 British towns between 1999 and 2001. Although adiponectin was associated with CAD risk factors in this study, adiponectin did not predict future risk of CAD [8]. Additional findings suggested that for each 1 μg/mL increase in plasma adiponectin level, there was an associated 3% risk reduction in CAD [9]. Lastly, a recent study demonstrated that plasma adiponectin levels in patients with acute coronary syndrome were significantly lower than those in patients with stable CAD [10]. Collectively these studies suggest that hypoadiponectinemia is a risk factor for development of atherosclerosis and CAD, underscoring the potential importance of this hormone.

Despite these associations, it is uncertain whether low adiponectin levels cause CAD. Genetic variants related to adiponectin levels can help us elucidate this relationship since they lie in the causal pathway and therefore their relationship with the outcome is likely not confounded by other risk factors. Further, since the temporal relationship between genetic variants and outcomes is clear, thereby excluding the possibility of reverse causation. Therefore, modern human genetics provides us with the opportunity to disentangle causal from non-causal associations between established risk factors including biomarkers such as adiponectin and CAD.

Variations in the gene encoding adiponectin (*ADIPOQ*) have been tested for their association with CAD. Two variants, rs2241766 (T+45G), rs1501299 (G+276T), have been assessed in several independent studies and a prospective analysis from Health Professionals follow-up study. While the rs1501299 variant showed evidence of association with CAD in Health Professionals Study [11] there no association was found in French/Swiss [12], Italian [13] and Japanese [14] studies. In addition, the rs2241766 variant was associated with CAD in the French/Swiss study [12] without showing evidence of association in Health Professional and Japanese studies [11,14]. Finally a coding variant, rs185847354, (I164T) had higher frequency among CAD cases in Japanese population [15]. Recent meta-analysis indicated a significant association of rs266729 (−11377C/G) but not rs2241766 or rs1501299 polymorphism with CAD among European and East Asian populations [16].

Several candidate and genome-wide association studies (GWAS) have shown pronounced associations between common polymorphisms in the *ADIPOQ*, *ARL15*, *CDH13* and *KNG1* and adiponectin levels [17–19]. Recently, in the largest multi-ethnic GWAS meta-analysis (n = 45,981) from ADIPOGen Consortium, we identified 10 novel loci for adiponectin levels and described their influence on risk of type 2 diabetes and metabolic traits [20].

In our ADIPOGen meta-analysis study, the coding and intronic variants in *STAB1* and *NT5DC2* were associated with weight to hip ratio (WHR) and high density lipoprotein cholesterol (HDL-C), while variants within 1 Mb of *TRIB1* were associated with all lipid traits. The coding and intronic variants in the locus on chromosome 12 harboring *ZNF664*, *CCDC92*, and *DNAH10* showed evidence of association with WHR, HDL-C, and TG. Finally, variants in the *PEPD* were associated with TG. [20]. This suggests that variants associated with adiponectin levels also correlated with components of the metabolic syndrome.

Given the lack of certainty regarding the nature of the relationship between adiponectin and CAD and the recent identification of replicated genetic determinants of adiponectin levels, we sought to determine whether the alleles that influence adiponectin levels also influence risk of CAD in the CARDIoGRAM consortium (n = 85,274).

2. Materials and methods

2.1. CAD samples

We used meta-analytic level data from the Coronary ARtery DIsease Genome-wide Replication And Meta-Analysis (CARDIoGRAM) consortium. Details of the design of CARDIoGRAM have been published previously [21]. Briefly, the CARDIoGRAM consortium combined GWAS data in individuals with European ancestry, including >22,000 cases with CAD, MI, or both and >60,000 controls, from 14 GWAS studies to understand the role of common genetic variation in CAD. The study was approved by correlated institutional review committees and that the subjects gave informed consent.

2.2. SNP selection

Through the ADIPOGen consortium we have recently conducted a meta-analysis of GWAS for adiponectin levels in European cohorts and in the joint analysis of discovery, in-silico and de-novo follow-up phases of study (n = 39 883) identified 162 SNPs to be genome-wide significant ($p < 5.0 \times 10^{-8}$) in their relationship with adiponectin levels [20]. (Table S1). These results include the previously described associations with adiponectin at *ADIPOQ* and *KNG1* on chromosome 3, and *CDH13* on chromosome 16. These analysis also identified novel loci on chromosome 1, 6, 8,12, 16, and 19. Details of genotyping methods and quality control criteria have been described previously [20,21].

2.3. Statistical analysis

Single-SNP analyses: We employed the Single Nucleotide Polymorphism Spectral Decomposition (SNPSpD) method that developed by Nyholt D [22]. This method corrects the multiple testing based on linkage disequilibrium (LD) using spectral decomposition matrices of pairwise LD between SNPs. Using this method, 145 SNPs were estimated to be equivalent to 113 independent statistical tests due to LD for their association. So we employed a Bonferroni-corrected threshold of $\alpha = 4.4 \times 10^{-4}$ (where $4.4 \times 10^{-4} = 0.05/113$) to define the threshold of association for any individual SNP association with CAD.

Multi-SNP Genotyping Risk Score: Using the 145 SNPs which were genome-wide significant for their relationship with adiponectin levels in cohorts of European ancestry from the ADIPOGen consortium, we selected LD-independent adiponectin associated alleles by LD pruning the set of genome-wide significant adiponectin SNPs