Adiponectin receptor 1 and small ubiquitin-like modifier 4 polymorphisms are associated with risk of coronary artery disease without diabetes

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Abstract

Background The genes encoding adiponectin receptor 1 (ADIPOR1) and small ubiquitin-like modifier 4 (SUMO4) have been linked to anti-atherogenic effects, but little is known about whether polymorphisms in the two genes, acting separately or interacting, affect risk of coronary artery disease (CAD) without diabetes. Methods We genotyped 200 CAD patients without diabetes and 200 controls without CAD or diabetes at three single-nucleotide polymorphisms (SNPs) in ADIPOR1 and one SNP in SUMO4, which were chosen based on previous studies. Potential associations were also explored between these SNPs and clinical characteristics of CAD without diabetes. Results Risk alleles at three SNPs in ADIPOR1 (rs7539542-G, rs7514221-C and rs3737884-G) and the G allele at SNP rs237025 in SUMO4 significantly increased risk of CAD without diabetes, with ORs ranging from 1.79 to 4.44. Carriers of any of these four risk alleles showed similar adverse clinical characteristics. Compared with individuals with a CC or GC genotype, those with a GG genotype at rs3737884 were at significantly higher risk of CAD that affected the left anterior descending coronary artery (OR: 6.77, \( P = 0.009 \)), the right coronary artery (OR: 4.81, \( P = 0.028 \)) or a relatively large number of vessels (\( P = 0.04 \)). Individuals carrying a risk allele at one or more of the three SNPs in ADIPOR1 as well as a risk allele at the SNP in SUMO4 were at significantly higher risk of CAD without diabetes than individuals not carrying any risk alleles (OR: 5.82, 95% CI: 1.23–27.7, \( P = 0.013 \)). Conclusions SNPs in ADIPOR1 and SUMO4 are associated with elevated risk of CAD without diabetes, and SNPs in the two genes may interact to jointly affect disease risk.

Keywords: Adiponectin receptor 1; Coronary artery disease; Diabetes; Polymorphism; Small ubiquitin-like modifier 4
case-control study in northern Han Chinese to examine possible associations between ADIPOR1 and SUMO4 SNPs and risk of CAD without diabetes. We also examined, for the first time systematically, whether the SNPs in ADIPOR1 and SUMO4 interact to jointly affect risk of CAD without diabetes.

2 Methods

2.1 Ethics statement

The Ethics Committee of Beijing Anzhen Hospital at Capital Medical University approved the study protocol. All participants provided informed consent in writing. The study was conducted according to the latest version of the Declaration of Helsinki.

2.2 Study population

A total of 400 subjects 65–75 years old were consecutively recruited from inpatients who had undergone coronary arteriography or coronary computed tomography angiography (CTA) for suspected or known coronary atherosclerosis at Beijing Anzhen Hospital between June 2013 and December 2014. All subjects were unrelated to one another and self-identified as Han Chinese living in Hebei or Shandong Province.

Cases (n = 200) were diagnosed with CAD for the first time based on ≥ 50% organic stenosis of at least one segment of a major coronary artery or its main branches, as confirmed by coronary angiography. All cases received standard treatment and none had relevant drug contraindications. Controls (n = 200) were age- and gender-matched inpatients at Beijing Anzhen Hospital who also self-identified as Han Chinese living in Hebei or Shandong Province and who met the following inclusion criteria: presence of < 40% organic stenosis by coronary angiography or coronary CTA; no evidence of CAD or myocardial infarction, as determined from medical records, ultrasonocardiography or electrocardiography according to relevant guidelines;[10] no diabetes or CAD in first-degree relatives; and no abnormal glucose tolerance. Controls were discharged from the hospital with no primary disease diagnosis (74, 37%) or with one of the following primary diagnoses: hyperlipidemia (49, 25%), hypertension (12, 6.0%), gastroesophageal reflux disease (61, 31%), atrial fibrillation (3, 1.5%), or sick sinus syndrome (1, 0.5%).

Individuals were excluded from the study if they met the criteria for acute ST segment elevation myocardial infarction (STEMI)[10] or diabetes based on World Health Organization criteria,[11] had a history of percutaneous coronary intervention,[10] or had a history of any of the following: acute heart failure, unstable hemodynamics, moderate or severe anemia, aortic dissection, myocardiopathy, congenital heart disease, secondary or uncontrolled hypertension, acute infection, severe liver or renal disease, autoimmune disease (including hyperthyroidism), malignancy, or immunosuppressive drug use.

2.3 Clinical and laboratory analyses

Clinical data were recorded on several factors potentially associated with CAD, including age, gender, systolic and diastolic blood pressure, body mass index (BMI), plasma levels of total cholesterol, triglycerides, fasting glucose, high- and low-density lipoprotein cholesterol, cigarette smoking, alcohol consumption, medication history and stenotic vessel characteristics.

2.4 SNP genotyping

We genotyped three SNPs in ADIPOR1 that in previous studies have shown a significant association with risk of CAD (OR > 1 and nominal \( P < 0.10 \))[5]: rs7539542 G > C, rs3737884 C > T, and rs7514221 C > T. We also genotyped one SNP in SUMO4 strongly suspected of being linked to diabetes and diabetes with CAD (rs237025 G > A).[6,7,12] Genomic DNA was extracted from whole blood samples using standard DNA isolation methods. SNPs were genotyped based on PCR-restriction fragment length polymorphism and high-resolution melting curve analysis as described.[5,12]

Five samples representing each of the observed genotypes at each SNP were randomly selected, amplified by PCR and sequenced by a third party to verify genotyping results. No discrepancies were observed. To minimize misclassification bias, researchers who genotyped SNPs were blinded to data about the study participants.

2.5 Statistical analysis

All statistical analyses were performed using SPSS 18.0 (IBM, Chicago, USA). Normally distributed data for continuous variables were expressed as mean ± SD, and mean values between groups were compared using an unpaired Student’s \( t \)-test. Skewed data for continuous variables were presented as median values and inter-quartile ranges and were compared between groups using the Mann-Whitney \( U \) test. Data for categorical variables were presented as percentages and were compared between groups using Pearson’s \( \chi^2 \) analysis or unconditional logistic regression. Genotype frequencies were compared between cases and
controls using the following genetic models: the additive model, which compares risk of CAD among individuals homozygous for the variant vs. heterozygous for the variant vs. homozygous for wild type; the dominance model, which compares risk between individuals homo- or heterozygous for the variant vs. homozygous for wild type; and the recessive model, which compares risk between individuals homozygous for the variant vs. heterozygous for the variant or homozygous for wild type. These comparisons were performed using unconditional logistic regression after adjusting for gender and BMI.

Tests for Hardy–Weinberg equilibrium (HWE) were performed separately for each SNP in controls and in cases using the online computer platform SHEsis (http://analysis.bio-x.cn/myAnalysis.php). Linkage disequilibrium (LD) analysis of the three ADIPOR1 SNPs was performed using Haploview 4.1 (Broad Institute, Cambridge, MA, USA). Possible gene–gene interactions were assessed using cumulative effect analysis and the multifactor dimensionality reduction (MDR) software package (GNU General Public License, Version 2). Strength of association between genotypes and risk of CAD was estimated using odds ratios (ORs) and 95% confidence intervals (95% CIs). All reported \(P\) values were two-sided, and the threshold of significance in all statistical tests was defined as \(P \leq 0.05\). Correction for multiple testing was performed using the Bonferroni method (http://www.quantitativeskills.com/sisa/calculations/bonfer.htm).

3 Results

3.1 Characteristics of the study population

Clinico-demographic characteristics of the study population are summarized in Table 1. Average age was 67.9 ± 3.3 years for cases, and 68.6 ± 3.6 years for controls. As expected, cases were significantly more likely than controls to have higher BMI, systolic and diastolic blood pressure, fasting blood glucose levels, total levels of triglycerides and total cholesterol, as well as lower levels of high-density lipoprotein cholesterol. Cases and controls showed similar incidence of cigarette use and alcohol consumption. According to the number of significantly affected vessels, cases were classified into three groups: 30 (15.0%) with one affected vessel, 75 (37.5%) with two affected vessels, and 95 (47.5%) with three affected vessels.

Since most cases had a relatively high BMI and since BMI varied less with drug history than other clinico-demographic characteristics, we included BMI as a covariate in subsequent association analyses.

Table 1. Clinical and demographic characteristics of Chinese patients with CAD without diabetes and of age- and gender-matched Chinese non-CAD control patients.

| Characteristic                           | Cases (n = 200) | Controls (n = 200) | \(P\) |
|------------------------------------------|----------------|-------------------|------|
| Age, yrs                                 | 67.9 ± 3.3     | 68.6 ± 3.6        | 0.79 |
| Male                                     | 138 (69.0%)    | 121 (60.5%)       | 0.08 |
| BMI, kg/m²                               | 25.3 ± 3.0     | 22.4 ± 2.6        | < 0.01 |
| SBP, mmHg                                | 130 (120–137)  | 110 (100–120)     | < 0.01 |
| DBP, mmHg                                | 80 (70–80)     | 70 (60–76)        | < 0.01 |
| FPG, mmol/L                              | 5.4 (4.9–6.3)  | 5.05 (4.8–5.3)    | < 0.01 |
| TG, mmol/L                               | 1.5 (1.1–2.1)  | 1.05 (0.8–1.4)    | < 0.01 |
| TC, mmol/L                               | 5.1 ± 1.0      | 4.4 ± 1.1         | < 0.01 |
| HDL-C, mmol/L                            | 1.0 (0.8–1.2)  | 1.6 (1.4–1.9)     | < 0.01 |
| LDL-C, mmol/L                            | 2.9 (2.4–3.6)  | 2.7 (2.3–3.3)     | 0.11 |
| Cigarette smoking*                       | 98 (49.0%)     | 81 (40.5%)        | 0.09 |
| Alcohol consumption*                     | 83 (41.5%)     | 79 (39.5%)        | 0.68 |
| History of hypertension                  | 127 (63.5%)    | 12 (12%)          | < 0.01 |
| History of hyperlipidemia                | 75 (37.5%)     | 49 (24.5%)        | < 0.01 |
| Previous myocardial infarction           | 0              | 0                 | < 0.01 |
| History of diabetes                      | 0              | 0                 | < 0.01 |
| Clinical diagnosis                       |                |                   |      |
| Stable angina pectoris                   | 21 (10.5%)     | 0                 | < 0.01 |
| Unstable angina pectoris                 | 154 (77%)      | 0                 | < 0.01 |
| Non-STEMI                                | 25 (12.5%)     | 0                 | < 0.01 |
| Number of affected vessels*              |                |                   |      |
| 1                                        | 30 (15.0%)     | 0                 | < 0.01 |
| 2                                        | 75 (37.5%)     | 0                 | < 0.01 |
| 3                                        | 95 (47.5%)     | 0                 | < 0.01 |
| History of medication                    |                |                   |      |
| Aspirin                                  | 200 (100%)     | 42 (21%)          | < 0.01 |
| P2Y12 receptor antagonist                | 200 (100%)     | 0                 | < 0.01 |
| Beta-blockers                            | 171 (85.5%)    | 2 (1%)            | < 0.01 |
| ACEI or ARB                              | 87 (43.5%)     | 0                 | < 0.01 |
| Statins                                  | 200 (100%)     | 42 (21%)          | < 0.01 |
| Calcium channel blockers                 | 39 (19.5%)     | 0                 | < 0.01 |
| Unfractionated heparin                   | 200 (100%)     | 0                 | < 0.01 |
| Low molecular weight heparin             | 25 (12.5%)     | 0                 | < 0.01 |
| GP IIb/IIIa inhibitors                   | 80 (40%)       | 0                 | < 0.01 |
| Warfarin                                 | 0              | 1 (0.5%)          | 0.5  |

Data are expressed as \(n\) (%), mean ± SD, or median (interquartile range).

*Current or previous; \(^*\)left main coronary artery stenosis was classified as affecting three vessels when the artery’s internal diameter had decreased by more than 50%. ACEI: angiotensin-converting enzyme inhibitors; ARB: angiotensin II receptor blockers; BMI: body mass index; CAD: coronary artery disease; DBP: diastolic blood pressure; FPG: fasting plasma glucose; GP IIb/IIIa inhibitors: glycoprotein IIb/IIIa receptor inhibitors. HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; SBP: systolic blood pressure; STEMI: ST-segment elevation acute myocardial infarction; TC: total cholesterol; TG: triglycerides.
3.2 ADIPOR1 and SUMO4 polymorphisms associated with CAD risk

Genotype frequencies at three SNPs in ADIPOR1 and one SNP in SUMO4 are shown in Table 2. The distribution of genotypes in cases and controls was consistent with HWE (P > 0.05). LD analysis of genotypes showed that D' was < 0.8 for all three SNPs in ADIPOR1, suggesting a lack of linkage disequilibrium (Figure 1).

Table 2. Genotype distributions at ADIPOR1 and SUMO4 SNPs in cases and controls.

| SNP    | Genotype | Genotype Count | Genotype Count | OR (95% CI) for each model | HWE |
|--------|----------|----------------|----------------|----------------------------|------|
|        |          | Cases          | Controls       | Additive                   |      |
|        |          |                |                | Dominant                   |      |
|        |          |                |                | Recessive                  |      |
|        |          |                |                | Cases                      |      |
|        |          |                |                | Controls                   |      |
| ADIPOR1| GG       | 90 (45.0%)     | 82 (41.0%)     | 1.34 (0.96–1.87)           |      |
|        | GC       | 92 (46.0%)     | 84 (42.0%)     | 1.50 (1.00–2.26)           |      |
|        | CC       | 18 (9.0%)      | 34 (17.0%)     | 3.45 (2.05–5.81)           |      |
|        | GG       | 130 (65.0%)    | 96 (48.0%)     | 1.95 (1.34–2.84)           |      |
|        | rs7539542| CC + GC        |                | 4.44 (1.64–12.00)          |      |
|        | rs754221 | CT + TT        |                | 1.96 (1.24–3.11)           |      |
|        | rs3737884| GA + AA        |                | 6.37×10⁻⁴                  |      |
|        | rs237025 | GA + AA        |                | 1.88 (1.31–2.71)           |      |
| SUMO4  | GG       | 26 (13.0%)     | 12 (6.0%)      | 1.88 (1.31–2.71)           |      |
|        | GA       | 86 (43.0%)     | 76 (38.0%)     | 2.11 (1.32–3.35)           |      |
|        | AA       | 88 (44.0%)     | 112 (56.0%)    | 2.63 (1.16–4.78)           |      |

*Risk genotype. HWE: Hardy-Weinberg equilibrium. ADIPOR1: adiponectin receptor 1; SUMO4: small ubiquitin-like modifier 4.

![Figure 1](http://www.jgc301.com)  
Figure 1. Linkage disequilibrium plots of three SNPs in the ADIPOR1 gene. (A): represents linkage disequilibrium measure of D’ in Controls; (B): D’ in Cases; (C): r² in Controls; (D): r² in Cases. ADIPOR1: adiponectin receptor 1; SUMO4: small ubiquitin-like modifier 4.

After adjusting for BMI, each of the three ADIPOR1 SNPs was found to be associated with increased risk of CAD: rs7539542-G, based on an additive model (OR: 3.45, 95% CI: 2.05–5.81, P < 0.001); rs7514221-C, based on an additive model (OR: 1.79, 95% CI: 1.10–2.91, P = 0.02) and a dominant model (OR: 2.03, 95% CI: 1.18–3.48, P = 0.01); and rs3737884-G, based on an additive model (OR: 1.95, 95% CI: 1.34–2.84, P < 0.001), a dominant model (OR: 4.44, 95% CI: 1.64–12.00, P = 0.003), and a recessive model (OR 1.96, 95% CI 1.24–3.11, P = 0.004).

In SUMO4, rs237025-G was found to be associated with increased risk of CAD after adjusting for BMI. This association appeared in an additive model (OR: 1.88, 95% CI: 1.31–2.71, P < 0.001), a dominant model (OR: 2.11, 95% CI: 1.64–12.00, P = 0.003), and a recessive model (OR 1.96, 95% CI 1.24–3.11, P = 0.004).

3.3 ADIPOR1 and SUMO4 polymorphisms associated with clinical manifestations of CAD

To further analyze the relationship between these SNPs and CAD phenotype, subjects carrying risk genotypes were divided into case and control subgroups and their clinical characteristics were compared (Table S1, data online). The following risk genotypes at each SNP were defined according to the dominant model (Table 2): ADIPOR1 rs7539542, GG + GC; ADIPOR1 rs7514221, CC + CT; ADIPOR1
rs3737884, GG + GA; and SUMO4 rs237025, GG + GA. Each of these risk genotypes was associated with a similar clinical phenotype (Table S1, data online). Among carriers of any of the four genotypes, cases were significantly more likely than controls to be male and to have higher BMI, systolic and diastolic blood pressure, fasting blood glucose levels, and total levels of triglycerides and cholesterol. Cases also showed significantly lower levels of high-density lipoprotein cholesterol.

Possible relationships between genotype distributions and CAD-affected vessels were also analyzed. None of the genotypes at the four SNPs was significantly associated with the primary vessel affected or with the number of vessels affected (data not shown), with one exception (Table 3). Comparing ADIPOR1 rs3737884 GG homozygotes with GA + AA individuals showed that disease in GG homozygotes was more likely to affect primarily the left main coronary artery (LM, 4.6% vs. 2.9%), the left anterior descending coronary artery (LAD, 53.8% vs. 42.9%) or the right coronary artery (RCA, 23.8% vs. 18.6%). Disease in GA+AA individuals was more likely to affect primarily the left circumflex coronary artery (LCX, 17.7% vs. 35.7%). In contrast to disease in GA + AA carriers, disease in GG homozygotes tended to affect more often the LAD (OR: 6.77, 95% CI: 1.25–5.16, \( P = 0.009 \)) or RCA (OR: 4.81, 95% CI: 1.10–6.13, \( P = 0.028 \)) (data not shown), although neither comparison achieved a \( P \) value below the Bonferroni significance threshold of 0.008. The GG genotype was more frequent than GA + AA genotypes in individuals with two affected vessels (57.3% vs. 42.7%) or three affected vessels (66.3% vs. 33.7%) (Table 3).

### 3.4 ADIPOR1-SUMO4 interaction and CAD risk

To explore possible combinations of genotypes that might affect the occurrence of CAD without diabetes, we performed cumulative effect analysis for each SNP in ADIPOR1 and SUMO4. This revealed that individuals carrying at least one risk allele at one or more of the three ADIPOR1 SNPs together with at least one risk allele at the SUMO4 SNP were at higher risk of CAD than individuals without any risk alleles at the four SNPs in this study (OR 5.82, 95% CI 1.23 to 27.7, \( P = 0.013 \)). In contrast, risk of CAD without diabetes for individuals with at least one risk allele at an ADIPOR1 SNP was similar to the risk for individuals without any risk alleles (OR 3.76, 95% CI 0.79 to 17.86, \( P = 0.076 \)). The risk was also similar for individuals with at least one risk allele at the SUMO4 SNP as for individuals without any risk alleles (OR 3.0, 95% CI 0.29 to 31.63, \( P = 0.35 \)) (Table 4).

We next used MDR software in an attempt to identify the best model capturing possible interactions among the four SNPs in this study. The best model showed a cross-validation consistency of 10/10 and accuracy of 58.9%. Nevertheless, it failed to identify any evidence of interaction between the ADIPOR1 and SUMO4 genes (testing \( \chi^2 = 1.28, P = 0.26 \); (Figure S1, data online).

### 4 Discussion

This case-control study in northern Han Chinese suggests that ADIPOR1 SNPs rs7539542-G, rs7514221-C, and rs3737884-G are positively associated with susceptibility to CAD without diabetes, confirming the associations...
reported in our previous study. Our study also provides the first evidence of an association between SUMO4 SNP rs237025-G and elevated risk of CAD without diabetes. Furthermore, we found that the combination of risk alleles in ADIPOR1 and SUMO4 increases risk of CAD without diabetes, suggesting that the two genes may interact to affect disease risk.

Our study was motivated in part by a report linking several common ADIPOR1 polymorphisms, including rs7539542 in the present work, with risk of CAD in patients with type 2 diabetes. Since diabetes and CAD are closely linked with each other in terms of pathogenesis and coexisting factors, we wanted to investigate the relationship between ADIPOR1 SNPs and risk of CAD without diabetes. Our results suggest that at least some ADIPOR1 SNPs influence risk of CAD independently of diabetes.

In previous work, we showed an association of ADIPOR1 rs3737884-G with elevated risk of CAD without diabetes in another Han group from northern China. In that work, as in the present study, we found evidence for an association of ADIPOR1 rs7539542-G and rs7514221-C with risk of CAD without diabetes, though the associated P values in the previous study did not meet the Bonferroni significance threshold for multiple testing (data not shown). Here P values remained significant even after correcting for multiple testing, probably reflecting the larger sample size and the fact that we applied stricter inclusion and exclusion criteria. Since that previous work and the present study involved non-overlapping populations, we combined the two datasets (n = 718) and repeated our association analyses. We found, as with each dataset by itself, that all three ADIPOR1 SNPs were significantly associated with CAD without diabetes (P < 0.05).

We found evidence that SUMO4 SNP rs237025-G is associated with risk of CAD without diabetes, extending a previous finding that it is also associated with CAD in Japanese patients with type 2 diabetes. Since the northern Han Chinese populations in our previous study and this one did not overlap, we combined both datasets (n = 1269) and confirmed that the SUMO4 rs237025-G is associated with elevated risk of CAD without diabetes based on a dominant genetic model (OR: 1.27, 95% CI: 1.02–1.59, P = 0.03).

An association between the SNP in SUMO4 and risk of CAD without diabetes is in accordance with biochemical evidence suggesting that SUMO4 functions as a negative feedback regulator of the NF-κB signaling pathway, helping control the potency of immune and inflammatory responses. SUMO4 modifies IκBα post-translationally, stabilizing it from degradation and allowing it to inhibit NF-κB. As a result, inactive NF-κB is sequestered in the cytoplasm, and this may help reduce the excessive inflammation characteristic of atherosclerosis in CAD. The rs237025-G variant leads to an amino acid substitution that weakens the ability of SUMO4 to inhibit NF-κB. This may explain why that allele is associated with higher risk of CAD.

Association between ADIPOR1 SNPs and risk of CAD without diabetes is consistent with observations that activating or up-regulating ADIPOR1 potentiates anti-inflammatory effects. In fact, it is possible that ADIPOR1 and SUMO4 overlap functionally in CAD, since ADIPOR1 inhibits TNF-α-induced degradation of IκBα, helping to keep NF-κB inactive. The three ADIPOR1 SNPs in the present study are located in introns, suggesting that they influence risk of CAD by acting as enhancers or silencers to alter ADIPOR1 transcription.

Given the possibility that ADIPOR1 and SUMO4 functionally interact in pathways affecting risk of CAD, we reasoned that SNPs in the two genes might exert a cooperative effect on risk of CAD in our population. Indeed, we observed indirect evidence of such interaction when we found that clinical manifestations of CAD without diabetes were similar across all risk genotypes at the four SNPs. We also found direct evidence of interaction: individuals with risk alleles at SNPs in both ADIPOR1 and SUMO4, but not individuals with risk alleles only in ADIPOR1 or only in SUMO4, were at higher risk of CAD without diabetes than individuals lacking any risk alleles at the four SNPs tested. However, we failed to identify gene-gene interactions using MDR software, which is reasonably strong at detecting such interactions in small samples. This negative result should be interpreted with caution. Genetic heterogeneity, which can arise from differences in genotypic penetrance and frequencies, severely limits the sensitivity of MDR analysis, as does the presence of strong main effects. It is possible that the two genes influence risk of CAD independently or that they interact directly through other SNPs that we did not examine. In any event, our data suggest that the association between ADIPOR1 SNPs and risk of CAD without diabetes (OR: 3.76) may be stronger than that between the SUMO4 SNP and disease risk (OR: 3.0) (Table 4).

By stratification, we found evidence that ADIPOR1 polymorphisms help determine the severity of CAD without diabetes. The primary affected vessel in patients carrying the rs3737884-G was most often in the LAD, followed by the RCA. Severe stenosis of the LAD causes serious ischemic events since it supplies such a large area of myocardium; it is the coronary vessel most often affected by atherosclerosis. It should be pointed out that CAD severity is determined by several factors besides the primary affected vessel; other factors include stenosis location, luminal di-
ameter, and plaque properties. As a first step to understanding whether SUMO4 and ADIPOR1 SNPs affect these aspects of CAD, we examined whether genotypes were associated with the number of affected vessels, which is often used to assess severity of atherosclerosis. Indeed, we found the GG genotype at rs3737884 in ADIPOR1 to be significantly associated with the number of affected vessels. We failed to find such an association in SUMO4, which may reflect the relatively small sample in our study. Since SUMO4 and ADIPOR1 are implicated in inflammatory responses, their SNPs may alter the stability of the plaque on the arterial wall, leading to plaque erosion and disruption.

Our study is subject to several limitations. While we included patients with unstable angina pectoris, we excluded patients with STEMI, which may bias our results. This decision was based on the fact that subjects with STEMI are at higher risk of sudden cardiac death with or without revascularization, which made it difficult to obtain their informed consent for this study. Another limitation of our study is that controls were inpatients, though care was taken to exclude anyone with objective evidence of CAD or myocardial infarction. Future studies should verify and extend our analyses in order to reveal more about the role of ADIPOR1 and SUMO4 polymorphisms and CAD and its severity.

In conclusion, the present study suggests that ADIPOR1 and SUMO4 SNPs are independently and jointly associated with risk of CAD without diabetes. One ADIPOR1 SNP was associated with stenosis phenotype, suggesting that it may be related to CAD severity. Further studies are needed to clarify whether and how ADIPOR1 and SUMO4 SNPs contribute to CAD susceptibility and severity.

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References

1. Ross R. Atherosclerosis—an inflammatory disease. N Engl J Med 1999; 340: 115–126.
2. Stern MP. Diabetes and cardiovascular disease. The "common soil" hypothesis. Diabetes 1995; 44: 369–374.
3. Yamauchi T, Nio Y, Maki T, et al. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. Nat Med 2007; 13: 332–339.
4. Wang CY, Yang P, Li M, et al. Characterization of a negative feedback network between SUMO4 expression and NFkappaB transcriptional activity. Biochem Biophys Res Commun 2009; 381: 477–481.
5. Jin Z, Pu L, Sun L, et al. Identification of susceptibility variants in ADIPOR1 gene associated with type 2 diabetes, coronary artery disease and the comorbidity of type 2 diabetes and coronary artery disease. PLoS One 2014; 9: e100339.
6. Shimada T, Furukawa Y, Furuta H, et al. SUMO4 Met55Val polymorphism is associated with coronary heart disease in Japanese type 2 diabetes individuals. Diabetes Res Clin Pract 2009; 85: 85–89.
7. Nosu S, Fujisawa T, Kawabata Y, et al. Association of small ubiquitin-like modifier 4 (SUMO4) variant, located in IDDM5 locus, with type 2 diabetes in the Japanese population. J Clin Endocrinol Metab 2007; 92: 2358–2362.
8. Pu LM, Nan N, Yang Z, Jin ZN. Association between SUMO4 polymorphisms and type 2 diabetes mellitus. Yi Chuan 2012; 34: 315–325. [Article in Chinese]
9. Soccio T, Zhang YY, Bacci S, et al. Common haplotypes at the adiponectin receptor 1 (ADIPOR1) locus are associated with increased risk of coronary artery disease in type 2 diabetics. Diabetes 2006; 55: 2763–2770.
10. Thygesen K, Alpert JS, Jaffe AS, et al. Third universal definition of myocardial infarction. Circulation 2012; 126: 2020–2035.
11. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998; 15: 539–553.
12. Pu LM, Nan N, Yang Z, et al. Association between SUMO4 polymorphisms and coronary artery disease with and without type 2 diabetes mellitus. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2012; 29: 596–601. [Article in Chinese]
13. Guo D, Li M, Zhang Y, et al. A functional variant of SUMO4, a new I kappa B alpha modifier, is associated with type 1 diabettes. Nat Genet 2004; 36: 837–841.
14. Zhang P, Wang Y, Fan Y, et al. Overexpression of adiponectin receptors potentiates the antiinflammatory action of subeffective dose of globular adiponectin in vascular endothelial cells. Arterioscler Thromb Vasc Biol 2009; 29: 67–74.
15. Coffey CS, Hebert PR, Ritchie MD, et al. An application of conditional logistic regression and multifactor dimensionality reduction for detecting gene-gene interactions on risk of myocardial infarction: the importance of model validation. BMC Bioinformatics 2004; 5: 49.
| Covariate | rs7539542 (GG + GC) | Controls (n = 166) | Z or t or $\chi^2$ | $P$ | rs7514221 (CC + CT ) | Controls (n = 39) | Z or t or $\chi^2$ | $P$ |
|-----------|---------------------|---------------------|---------------------|-----|---------------------|---------------------|---------------------|-----|
| Age, yrs  | 60.5 (52–68)        | 63 (48–70)          | 0.37                | 0.71| 59.2 ± 11.2         | 58.4 ± 13.0         | −0.311              | 0.757 |
| Male      | 131 (72.0%)         | 92 (55.4%)          | 10.34               | 1.30×10⁻³| 42 (72.4%)         | 17 (43.6%)          | 8.13                | 4.35×10⁻³ |
| BMI, kg/m²| 25.3 ± 3.0          | 22.3 ± 2.6          | −9.82               | 3.19×10⁻³| 25.1 (22.9–27.4)   | 21.4 (21–22.7)      | −5.45               | 4.97×10⁻³ |
| ≤ 22.9    | 39 (21.4%)          | 100 (60.2%)         | 74.32               | 7.2×10⁻¹⁷| 15 (25.9%)         | 30 (76.9%)          | 29.20               | 4.57×10⁻⁷ |
| ≥ 25      | 94 (51.6%)          | 20 (12.0%)          |                     |     |                     |                     |                     |     |
| SBP, mmHg | 130 (120–140)       | 110 (100–120)       | −10.16              | 2.85×10⁻²⁴| 125.3 ± 13.7       | 111.6 ± 9.6        | −5.40               | 4.88×10⁻⁷ |
| DBP, mmHg | 80 (70–82)          | 70 (60–76)          | −6.45               | 1.14×10⁻²⁰| 76.7 ± 9.9         | 68.8 ± 7.4         | −4.27               | 4.62×10⁻⁴ |
| FBG, mmol/L| 5.4 (4.9–6.3)       | 5.1 (4.8–5.3)       | −5.43               | 5.62×10⁻⁸ | 5.4 (4.9–6.2)      | 5.1 (4.9–5.4)      | −2.29               | 0.02  |
| TG, mmol/L| 1.5 (1.1–2.2)       | 1.0 (0.8–1.3)       | −7.68               | 1.61×10⁻¹⁴| 1.5 (1.0–2.0)      | 1.0 (0.8–1.3)      | −4.00               | 6.26×10⁻⁸ |
| TC, mmol/L| 5.1 ± 0.9           | 4.4 ± 1.1           | 6.36                | 6.47×10⁻¹⁰| 1.7 ± 1.0          | 1.0 ± 0.3          | 3.46                | 8.11×10⁻⁴ |
| HDL-C, mmol/L| 0.9 (0.8–1.2)      | 1.6 (1.4–1.8)       | −14.15              | 1.74×10⁻¹⁰| 0.9 ± 0.2          | 1.7 ± 0.3          | 13.47               | 9.28×10⁻¹⁰ |
| LDL-C, mmol/L| 2.7 (2.1–3.5)      | 2.7 (2.3–3.3)       | −0.38               | 0.705| 2.8 (2.0–3.6)      | 2.6 (2.4–3.4)      | −0.73               | 0.45  |

| Covariates | rs3737884 (GG + GA ) | Controls (n = 177) | Z or t or $\chi^2$ | $P$ | rs237025 (GG + GA ) | Controls (n = 88) | Z or t or $\chi^2$ | $P$ |
|------------|---------------------|---------------------|---------------------|-----|---------------------|---------------------|---------------------|-----|
| Age, yrs   | 51.5 (60–67.5)      | 62 (48–70)          | −0.47               | 0.64| 59.6 ± 10.9         | 59.1 ± 12.3         | −0.31               | 0.76  |
| Male       | 139 (72.0%)         | 98 (55.4%)          | 11.12               | 8.54×10⁻⁴ | 83 (74.1%)        | 42 (47.7%)          | 14.63               | 1.31×10⁻⁴ |
| BMI, kg/m² | 25.2 ± 3.0          | 22.4 ± 2.6          | −9.71               | 5.40×10⁻¹⁰| 24.9 (23.1–26.6)  | 21.9 (20.9–24.0)   | −6.49               | 8.50×10⁻¹⁵ |
| ≤ 22.9     | 42 (21.8%)          | 104 (58.8%)         | 76.53               | 2.41×10⁻¹⁷| 27 (24.1%)        | 57 (64.8%)          | 42.11               | 7.17×10⁻⁹  |
| ≥ 25       | 101 (52.3%)         | 22 (12.4%)          |                     |     |                     |                     |                     |     |
| SBP, mmHg  | 129 (120–137.5)     | 110 (100–120)       | −10.08              | 7.11×10⁻¹⁴| 124 (120–130)    | 110 (100–120)      | −6.9                | 3.92×10⁻¹² |
| DBP, mmHg  | 80 (70–86)          | 70 (60–77)          | −6.26               | 3.79×10⁻¹⁰| 75.5 (70–80)      | 70 (60–76)         | −4.3                | 1.89×10⁻⁴ |
| FBG, mmol/L| 5.4 (4.9–6.3)       | 5.0 (4.8–5.3)       | −7.59               | 8.41×10⁻⁸ | 5.3 (4.9–6.1)     | 5.1 (4.8–5.3)      | −3.48               | 5.05×10⁻⁴ |
| TG, mmol/L | 1.5 (1.1–2.1)       | 1.1 (0.8–1.4)       | −7.59               | 3.29×10⁻¹⁴| 1.5 (1.1–2.3)     | 1.0 (0.8–1.4)      | −5.71               | 1.14×10⁻⁴ |
| TC, mmol/L | 5.1 ± 1.0           | 4.4 ± 1.1           | 6.48                | 2.90×10⁻¹⁰| 5.1 ± 1.0         | 4.32 ± 1.1         | 5.08                | 8.53×10⁻⁷  |
| HDL-C, mmol/L | 0.9 (0.7–1.0)     | 1.6 (1.4–1.8)       | −14.61              | 2.34×10⁻⁹ | 0.9 ± 0.2         | 1.6 ± 0.3          | 16.79               | 6.14×10⁻⁹  |
| LDL-C, mmol/L | 2.7 (2.1–3.5)     | 2.7 (2.3–3.3)       | −0.37               | 0.71 | 2.6 (2.1–3.4)     | 2.7 (2.3–3.4)      | −1.13               | 0.26  |

Data are expressed as n (%), mean ± SD, or median (interquartile range). Risk genotypes were defined on the basis of the genetic dominance model analysis in Table 2. ADIPOR1: adiponectin receptor 1; BMI: body mass index; CAD: coronary artery disease; DBP: diastolic blood pressure; FPG: fasting plasma glucose; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; SBP: systolic blood pressure; SUMO4: small ubiquitin-like modifier 4; TC: total cholesterol; TG: triglycerides.
Figure S1.  Interaction between the *ADIPOR1* and *SUMO4* genes on risk of coronary artery disease. Red lines connecting SNPs indicate synergistic interaction; blue lines, antagonistic interaction; and green lines, no interaction. *ADIPOR1*: adiponectin receptor 1; *SUMO4*: small ubiquitin-like modifier 4.