Circulating serum amyloid A levels but not SAA1 variants predict long-term outcomes of angiographically confirmed coronary artery disease

Kuan-Hung Yeh\textsuperscript{a,b}, Lung-An Hsu\textsuperscript{c}, Jyh-Ming Jimmy Juang\textsuperscript{d}, Fu-Tien Chiang\textsuperscript{e}, Ming-Sheng Teng\textsuperscript{f}, I-Shiang Tzeng\textsuperscript{g}, Semon Wu\textsuperscript{e}, Jeng-Feng Lin\textsuperscript{a,b}, Yu-Lin Ko\textsuperscript{a,b,g}*

**Abstract**

**Objectives:** Circulating serum amyloid A (SAA) levels are strongly associated with atherosclerotic cardiovascular disease risk and severity. The association between SAA1 variants, SAA levels, inflammatory marker levels, and coronary artery disease (CAD) prognosis has not been fully understood. **Materials and Methods:** In total, 2199 Taiwan Biobank (TWB) participants were enrolled for a genome-wide association study (GWAS), and the long-term outcomes in 481 patients with CAD were analyzed. The primary endpoint was all-cause mortality, and the secondary endpoint was the combination of all-cause death, myocardial infarction, stroke, and hospitalization for heart failure.

**Results:** Through GWAS, SAA1 rs11024600 and rs7112278 were independently associated with SAA levels ($P = 3.84 \times 10^{-14}$ and $P = 1.05 \times 10^{-29}$, respectively). SAA levels were positively associated with leukocyte counts and multiple inflammatory marker levels in CAD patients and with body mass index, hemoglobin, high-density lipoprotein cholesterol, and alanine aminotransferase levels in TWB participants. By stepwise linear regression analysis, SAA1 gene variants contributed to 27.53% and 8.07% of the variation of the SAA levels in TWB and CAD populations, respectively, revealing a stronger influence of these two variants in TWB participants compared to CAD patients. Kaplan–Meier survival analysis revealed that SAA levels, but not SAA1 gene variants, were associated with long-term outcomes in patients with CAD. Cox regression analysis also indicated that high circulating SAA levels were an independent predictor of both the primary and secondary endpoints. **Conclusion:** SAA1 genotypes contributed significantly to SAA levels in the general population and in patients with CAD. Circulating SAA levels but not SAA1 genetic variants could predict long-term outcomes in patients with angiographically confirmed CAD.

**Keywords:** Coronary artery disease, Genome-wide association study, Long-term outcomes, SAA1 gene, Serum amyloid A

**Introduction**

The serum amyloid A (SAA) family of apolipoproteins is a group of sensitive biomarkers that characterize the inflammatory state [1]. Although SAA is secreted under normal physiological conditions from a low-level SAA4 expression, its levels can increase a thousandfold.

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: WKHLPFMedknow_reprints@wolterskluwer.com

How to cite this article: Yeh KI, Hsu IA, Juang JM, Chiang FT, Teng MS, Tzeng IS, et al. Circulating serum amyloid A levels but not SAA1 variants predict long-term outcomes of angiographically confirmed coronary artery disease. Tzu Chi Med J 2022;34(4):423-33.
when induced by inflammatory stimuli during acute-phase reactions, predominantly from the increased \( SAA1 \) and \( SAA2 \) expression [1,2]. Inducible expression of \( SAA \) is a hallmark of the acute-phase response, which is a reaction in vertebrates to protect against environmental challenges such as tissue injury, infection, and surgery [3]. In addition to being released by activated macrophages on infection, traumatic injury, or stress, \( SAA \) is mainly secreted by hepatocytes and various other cells such as vascular smooth muscle cells and endothelial cells following interleukin-1 and interleukin-6 stimulation [4,5].

On release, \( SAA \) promotes monocyte and neutrophil chemotaxis and participates in high-density lipoprotein cholesterol (HDL-C) metabolism and transportation and innate immune response [6-8]. \( SAA \) is also an exchangeable apolipoprotein that increases binding of apoB-containing lipoprotein to proteoglycan in both mice and humans [9]. \( SAA \) has been shown to be associated with a number of pro-inflammatory and pro-atherogenic activities, and appears to play a causal role in atherogenesis [6,10-13]. Furthermore, \( SAA \) proteins interact with specific receptors and have been implicated in multiple physiological and pathophysiologically processes, such as tissue remodeling, maternal–fetal health, intestinal physiology, inflammatory rheumatic diseases, lung inflammation, and cancer metastasis [14,15]. By meta-analysis, Zhou et al. [16] showed that high levels of \( SAA \) are positively associated with C-reactive protein (CRP), fibrinogen, and interleukin 6 levels in patients with coronary artery disease (CAD). In addition, elevated \( SAA \) levels are strongly indicative of atherosclerotic lesion and cardiovascular disease event risk and severity [16-20].

A substantial part of \( SAA \) levels is determined by genetic variation. For instance, the 49%–67% of heritability estimates of baseline \( SAA \) levels were derived from studies of twins [21]. In a genome-wide association study (GWAS), two genetic susceptibility regions, 1p15.5-p13 and 1p31, were identified, with 1p31 containing \( SAA \) family genes and contributing to 18.4% of the total estimated heritability of \( SAA \) levels [22]. We previously confirmed a highly significant association between the \( SAA1 \) single-nucleotide polymorphism (SNP) rs4638289 and levels of both \( SAA \) and inflammatory marker CRP in a Taiwanese cohort undergoing cardiovascular health examination [23]. Whether genetic determinants of \( SAA \) levels can predict atherosclerotic cardiovascular disease prognosis remains unknown.

In this study, we investigated the genetic determinants of \( SAA \) levels in Taiwanese people through a GWAS from the participants of the Taiwan Biobank (TWB) population. The effect of the combination of \( SAA \) and CRP levels and the associations of \( SAA1 \) genotypes/weighted genetic risk scores (WGRSs) with \( SAA \) levels and the long-term outcomes in patients with CAD were further analyzed.

**Materials and Methods**

**Participants**

The GWAS cohort comprised participants from the TWB population. Information was gathered at recruitment centers across Taiwan between 2008 and 2015. In total, 2349 participants with no history of cancer, stroke, CAD, or systemic diseases were recruited. Of these, 150 participants were excluded from analysis because they withdrew their informed consent (\( n = 2 \), fasted for <6 h (\( n = 38 \)), or failed quality control (QC) for GWAS (\( n = 110 \)). Finally, 2199 participants were enrolled for the analysis. Ethical approval was received from the Research Ethics Committee of Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation (approval number: 05-X04-007), and the Ethics and Governance Council of the TWB (approval numbers: TWBR10507-02 and TWBR10611-03).

Between July 2010 and September 2013, 483 patients with CAD who presented with >50% stenosis in one major coronary artery in the coronary angiography performed at National Taiwan University Hospital were enrolled for analysis. The blood samples were collected during coronary angiography after written informed consent was obtained. Two more patients were excluded because they were not followed up after their initial hospitalization, and 481 patients were ultimately enrolled. The primary endpoint was all-cause mortality, and the secondary endpoint was a combination of all-cause death, myocardial infarction, stroke, and hospitalization for heart failure. A flowchart of the enrolment for TWB participants and CAD patients with the inclusion and exclusion algorithm is shown in Figure 1. Relevant ethical approval was obtained from the Institutional Review Board of National Taiwan University Hospital (approval number: 201002015M).

**Genome-wide association study**

Axiom Genome-Wide CHB 1 Array plate (Affymetrix Inc., Santa Clara, CA, USA), was used as the genotype array for GWAS in TWB using Armitage trend test (implemented in PLINK). QC for the GWAS was performed and 2199 participants and 614,821 SNPs were enrolled for GWAS analysis after QC [Supplementary Method].**

**DNA genotyping**

For the TWB participants, SNP genotyping was conducted using the custom TWB chips on the Axiom Genome-Wide Array Plate System (Affymetrix). For the patients with CAD, genotyping was completed using the TaqMan SNP Genotyping Assay Kits (Applied Biosystems, Foster City, CA, USA) [24,25].

**Clinical and biochemical analysis**

Clinical phenotypes used for analysis included body height and weight, body mass index (BMI), waist circumference, waist-to-hip ratio, and systolic and diastolic blood pressure. Biochemistry data enrolled for analysis included fasting plasma glucose levels; hemoglobin A1C (HbA1C) levels; lipid profile including total cholesterol, HDL, low-density lipoprotein cholesterol, and triglyceride levels; and serum creatinine, uric acid, aspartate aminotransferase, alanine aminotransferase (ALT), γ-glutamyl transferase, albumin, and total bilirubin levels. BMI was calculated as body weight (in kg)/height (in m²). The estimated glomerular filtration rate (eGFR) was calculated using the following equation: 175× serum creatinine\(^{-1.154}\) × age\(^{-0.203}\) (× 0.742 for female patients) [26]. The levels of inflammatory markers,
including serum SAA, soluble intercellular adhesive molecule 1 (sICAM1), sE-selectin, and matrix metalloprotease 9 (MMP9), were measured using commercially available sandwich enzyme-linked immunosorbent assay (ELISA) kits from R&D (Minneapolis, MN, USA) for the patients with CAD [27]. Circulating plasma levels of CRP were measured using the particle-enhanced turbidimetric immunoassay technique (Siemens Healthcare Diagnostics Ltd., Camberley, UK) in CAD patients. The intra- and inter-assay variability of the studied inflammatory markers was within the range of 2.3% to 7.9% [Supplementary Table 1].

Statistical analysis

Normally distributed continuous variables were compared using the two-sample t-test or one-way analysis of variance. The means and their standard deviations (SDs) were suggested for distributions that satisfied parametric assumptions. The medians and interquartile ranges (IQRs) were suggested for skewed data or data with outliers. Associations among the categorical variables were analyzed using Pearson’s Chi-squared test. Associations between comparisons of skewed data were analyzed using Mann–Whitney U-test. Associations between SAA levels of SAA genotyping were analyzed using Kruskal–Wallis test. Fasting SAA levels and all other studies parameters were transformed using the logarithmic function if they violated the assumption of normality. The general linear model was used to conduct multiple regression analysis to determine the association between SAA levels and the predictors of investigated genotypes. The genetic effect was estimated through multiple linear regression analysis and adjustment for age, sex, BMI, and current smoking status. The WGRS was calculated by multiplying the estimated beta-coefficient of each SNP by the number of corresponding risk alleles (0, 1, or 2). We also compared the SAA levels to predict primary and secondary endpoints by plotting receiver operating characteristic (ROC) curves. The survival curve was plotted using the Kaplan–Meier method, and the significance was examined using the log-rank test. Statistical analyses were performed using SPSS (version 22.0; SPSS, Chicago, IL, USA). All tests were two-tailed, and P < 0.05 indicated statistically significant differences and associations. Missing data were approached through pairwise deletion.

For GWAS, genetic association analysis was performed using PLINK. We used the conventional threshold of \( P < 5 \times 10^{-8} \) (i.e., Bonferroni correction for 1 million tests) for genome-wide statistical significance. Conditional analysis were performed using SPSS (version 22.0; SPSS, Chicago, IL, USA). All tests were two-tailed, and \( P < 0.05 \) indicated statistically significant differences and associations. Missing data were approached through pairwise deletion.

Results

Association of serum amyloid A levels with clinical, biochemical, and hematological phenotypes in Taiwan Biobank participants

In the TWB participants, the median SAA level was 8.01 μg/mL (IQR: 4.33–14.20 μg/mL), and the association between SAA level and clinical, biochemical, and hematological phenotypes is presented in Table 1 and Supplementary Table 2. The SAA level was significantly associated with age, sex, BMI, and other traits. Further, after adjustments for age, sex, BMI, and smoking with Bonferroni correction, the SAA levels presented positive associations with HDL-C and ALT levels, hemoglobin, leukocyte counts, and triglyceride levels.

Genome-wide association study of serum amyloid A levels in Taiwan Biobank participants

For GWAS, we fitted a linear regression model with genotype trend effects and adjusted it for age, sex, BMI, and smoking status. The peak − log₁₀ \( P \) for circulating SAA levels was observed in chromosome 11p15.5-p13, where
Table 1: Association between circulating serum amyloid A levels and clinical, biochemical, and hematological parameters in Taiwan Biobank participants

| Clinical, biochemical, and hematological parameters     | n     | Median (IQR)     | r      | P     | r      | P      | Adjusted P |
|--------------------------------------------------------|-------|------------------|--------|-------|--------|--------|------------|
| Anthropology                                           |       |                  |        |       |        |        |            |
| Age (years)                                            | 2199  | 48.0 (39.0-57.0) | 0.078  | 2.63×10⁻⁴ | 0.0171 | 7.71×10⁻⁴ | 0.0185     |
| Waist circumference (cm)                               | 2199  | 83.0 (77.0-89.8) | 0.153  | 6.30×10⁻¹³ | 0.0440 | 0.0391 | 0.9337     |
| Waist hip ratio                                        | 2199  | 0.8±0.1          | 0.1    | 3.00×10⁻⁶  | 0.0326 | 0.1270 | 0.9999     |
| BMI (kg/m²)                                            | 2199  | 23.9 (21.7-26.1) | 0.17   | 4.41×10⁻¹⁴ | 0.1922 | 1.02×10⁻¹⁴ | 2.44×10⁻¹⁸ |
| BP (mmHg)                                              |       |                  |        |       |        |        |            |
| Systolic BP                                            | 1996  | 110.0 (101.1-121.9) | 0.076  | 0.001 | 0.0116 | 0.6035 | 0.9999     |
| Diastolic BP                                           | 1996  | 70.0 (62.0-77.3) | 0.07   | 0.002 | 0.0299 | 0.1816 | 0.9999     |
| Mean BP**                                              | 1996  | 83.3 (76.3-91.7) | 0.077  | 0.001 | 0.0242 | 0.2805 | 0.9999     |
| Lipid profiles (mg/dL)                                 |       |                  |        |       |        |        |            |
| Total cholesterol¹                                      | 2104  | 192.0 (170.0-217.0) | 0.094  | 1.40×10⁻¹  | 0.0640 | 3.34×10⁻¹  | 0.0802     |
| HDL-cholesterol²                                       | 2104  | 54.0 (45.0-64.0) | 0.044  | 0.043 | 0.0862 | 7.60×10⁻¹  | 1.82×10⁻³   |
| LDL-cholesterol²                                       | 2104  | 120.0 (99.0-143.0) | 0.072  | 0.001 | 0.0406 | 0.0627 | 0.9999     |
| Triglyceride²                                          | 2104  | 94.0 (66.0-134.0) | 0.055  | 0.011 | 0.0014 | 0.9483 | 0.9999     |
| Glucose metabolism                                     |       |                  |        |       |        |        |            |
| Fasting plasma glucose (mg/dL)**                       | 2104  | 92.0 (87.0-97.0) | 0.065  | 0.003 | 0.0384 | 0.0787 | 0.9999     |
| HbA1C (g/dL)**                                         | 2104  | 5.6 (5.3-5.8)    | 0.073  | 0.001 | 0.0293 | 0.1792 | 0.9999     |
| UA (mg/dL)**                                           | 2109  | 5.3 (4.5-6.3)    | 0.027  | 0.218 | 0.0259 | 0.2348 | 0.9999     |
| Renal function                                         |       |                  |        |       |        |        |            |
| Creatinine (mg/dL)                                     | 2199  | 0.7 (0.6-0.9)    | -0.003 | 0.905 | 0.0397 | 0.0631 | 0.9999     |
| eGFR (mL/min/1.73 m²)                                  | 2199  | 88.4 (78.5-99.5) | -0.058 | 0.006 | -0.0419 | 0.0499 | 0.9999     |
| Liver function                                         |       |                  |        |       |        |        |            |
| AST (U/L)                                              | 2199  | 21.0 (18.0-26.0) | 0.071  | 0.001 | 0.0526 | 0.0137 | 0.3286     |
| ALT (U/L)                                              | 2199  | 19.0 (14.0-27.0) | 0.111  | 1.61×10⁻⁷ | 0.0920 | 1.60×10⁻⁷ | 3.84×10⁻⁴   |
| γGT (U/L)                                              | 2199  | 18.0 (12.0-27.0) | 0.056  | 0.008 | 0.0517 | 0.0153 | 0.3681     |
| Serum albumin (g/dL)                                   | 2199  | 4.6 (4.5-4.8)    | -0.066 | 0.002 | -0.0305 | 0.1538 | 0.9999     |
| Total bilirubin (mg/dL)                                | 2199  | 0.6 (0.5-0.8)    | -0.072 | 0.001 | -0.0492 | 0.0212 | 0.5083     |
| Hemogram                                               |       |                  |        |       |        |        |            |
| Leukocyte count (10⁹/μL)                               | 2199  | 5.9 (5.0-7.0)    | 0.135  | 2.05×10⁻¹⁰ | 0.1173 | 3.61×10⁻¹⁰ | 8.65×10⁻⁷   |
| Hemoglobin (g/dL)                                      | 2199  | 14.0 (13.0-15.1) | 0.025  | 0.241 | 0.0685 | 0.0013 | 0.0315     |
| Platelet count (10⁹/μL)                                | 2199  | 235.0 (201.0-273.0) | 0.029 | 0.171 | 0.0079 | 0.7100 | 0.9999     |

¹P value: Unadjusted, ²P value: Adjusted for sex, age, BMI, smoking status, ³Adjusted P value, adjusted for sex, age, BMI, smoking status with Bonferroni correction (n=24), ⁴Were analyzed with the exclusion of participants with previous history of hypertension, ⁵Were analyzed with the exclusion of participants with previous history of diabetes mellitus, ⁶Were analyzed with the exclusion of participants with previous history of gout, ⁷Were analyzed with the exclusion of participants with previous history of hyperlipidemia. Data are presented as median (IQR) or mean±SD. BP: Blood pressure, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, HbA1C: Hemoglobin A1C, AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, eGFR: Estimated glomerular filtration rate, GGT: γ-Glutamyl transferase, IQR: Interquartile range, SD: Standard deviation, BMI: Body mass index, UA: Uric acid

SAA1 is located [Figure 2a and b]. No other chromosome loci reached genome-wide significance. Initially, 16 SNPs exceeded the genome-wide significance threshold (P < 5 × 10⁻⁸; Supplementary Table 3). The linkage disequilibrium among the 16 SAA1 SNPs is illustrated in Supplementary Figure 1. The top-hit SNP was SNP rs11024600 (P = 3.84 × 10⁻¹⁴), located 3' downstream from SAA1 [Figure 2(b) and Table 2]. To clarify whether the association of other SAA1 SNPs was independent of the lead SNP, we performed stepwise conditional analysis by using the GWAS samples. After adjustment of the rs11024600 genotypes, rs7112278 in the regional plot at the SAA1 locus remained genome-wide significant with SAA levels (P = 1.05 × 10⁻²⁹; Figure 2(c)). After the GWAS was adjusted for the two SNPs, no other SNP in the regional plot near the SAA1 locus exhibited significance at P < 0.001 [Figure 2d]. The quantile–quantile plot indicated the observed significant associations beyond those expected by chance [Figure 2e]. A more significant association was noted when the WGRSs combined with the two SNPs were enrolled for the analysis [Table 3]. Genetic variants of the leptin receptor gene (LEPR) were associated with SAA levels in a previous GWAS report [22]. We tested whether this association was present in our population; however, none of the SNPs within 20 kb of the LEPR gene region reached a P < 0.001 [Supplementary Figure 2].

Associations between serum amyloid A1 genotypes and serum amyloid A levels in patients with coronary artery disease

In patients with CAD, the three SAA1 SNPs rs11024600, rs7112278, and rs4638289 were genotyped using the Taqman assay. The SNPs rs4638289 and rs7112278 presented a nearly complete linkage disequilibrium (r = 0.995). Our data revealed a significant and independent association between individual genotyping or WGRS of rs11024600 and rs7112278 and the SAA levels in patients with CAD [Table 3].
Table 2: Clinical and biochemical characteristics of the coronary artery disease patients according to their survival state

| Baseline characteristics                  | Total (481) | Without secondary end point (427) | With secondary end point (54) | P       |
|-------------------------------------------|-------------|-----------------------------------|-----------------------------|---------|
| Sex (male/female)                         | 388/93      | 351/76                            | 37/17                       | 0.0160  |
| Age (years)                               | 66.0 (58.0-74.0) | 65.0 (57.0-73.0) | 73.0 (65.0-80.0) | 5.05×10^-4 |
| Body mass index (kg/m^2)                  | 25.6 (23.5-28.3) | 25.7 (23.5-28.4) | 25.1 (22.7-27.5) | 0.0106  |
| Hypertension, n (%)                       | 376 (78.2) | 330 (77.3)                       | 46 (85.2)                   | 0.1850  |
| Diabetes mellitus, n (%)                  | 213 (44.3) | 179 (41.9)                       | 34 (63.0)                   | 0.0030  |
| Dyslipidemia, n (%)                       | 293 (60.9) | 268 (62.8)                       | 25 (46.3)                   | 0.0630  |
| Current smoker, n (%)                     | 117 (24.3) | 108 (25.3)                       | 9 (16.7)                    | 0.1640  |
| Initial presentation, n (%)               |             |                                   |                             |         |
| Stable angina pectoris                    | 405 (84.2) | 380 (89)                          | 25 (46.3)                   | 2.03×10^-16 |
| ACS/Lung edema                            | 37 (7.7)   | 22 (5.2)                          | 15 (27.8)                   |         |
| CHF/Lung edema                            | 22 (4.6)   | 11 (2.6)                          | 11 (20.4)                   |         |
| Others                                    | 17 (3.5)   | 14 (3.3)                          | 3 (5.6)                     |         |
| CAD (S vs. D vs. T)                       | 134 (27.9): 135 (28.1): 212 (44.1) | 130 (30.4): 122 (28.6): 175 (41.0) | 4 (7.4): 13 (24.1): 37 (68.5) | 1.42×10^-4 |
| Biochemistry                               |             |                                   |                             |         |
| Serum creatinine (mg/dL)                  | 1.1 (0.9-1.3) | 1.0 (0.9-1.2) | 1.3 (1.9-2.1) | 8.95×10^-4 |
| eGFR (mL/min/1.73m²)                      | 67.8 (54.3-80.7) | 68.5 (55.6-81.7) | 51.7 (29.7-68.4) | 1.29×10^-4 |
| Blood cell counts                         |             |                                   |                             |         |
| Leukocyte counts (10^9/L)                 | 6.3 (5.3-7.7) | 6.2 (5.3-7.5) | 7.2 (5.2-8.9) | 0.0516  |
| Hematocrit (%)                            | 41.8 (38.1-44.7) | 41.9 (38.8-44.8) | 36.8 (31.0-43.1) | 2.64×10^-4 |
| Platelet counts (10^9/L)                  | 205.0 (170.0-241.5) | 206.0 (178.0-241.8) | 179.0 (137.0-248.0) | 0.0122  |
| Inflammation markers                      |             |                                   |                             |         |
| CRP (mg/L)                                | 2.5 (1.2-4.3) | 2.3 (1.2-3.9) | 4.2 (2.0-16.8) | 1.16×10^-3 |
| SAA (μg/mL)                               | 11.2 (5.4-27.6) | 10.2 (5.3-24.8) | 21.1 (9.0-287.9) | 5.00×10^-3 |
| sICAM1 (ng/mL)                            | 126.4 (107.6-149.4) | 123.9 (106.4-144.2) | 150.3 (123.0-178.8) | 3.27×10^-4 |
| sE-selectin (ng/mL)                       | 10.8 (8.3-14.0) | 10.7 (8.3-13.8) | 11.8 (7.4-16.6) | 0.1886  |
| MMP9 (ng/mL)                              | 75.2 (47.7-115.3) | 73.4 (47.1-114.4) | 81.5 (53.5-123.8) | 0.2786  |

P value: Unadjusted (Mann–Whitney U test). Data are presented as percentage, or median (IQR) as appropriate. ACS/MI: Acute coronary syndrome or myocardial infarction, CHF: Congestive heart failure, S versus D versus T: Single versus double versus triple vessel, CAD: Coronary artery disease, eGFR: Estimated glomerular filtration rate, SAA: Serum amyloid A, sICAM1: Soluble intercellular adhesive molecule, sE-selectin: Soluble E-selectin, MMP9: Matrix metalloprotease 9, IQR: Interquartile range, CRP: C-reactive protein

Table 3: Association between serum amyloid A genotypes and weighted genetic risk scores and serum amyloid A levels

| Genotypes/WGRS | Population | MM | Mn | mm | P | β | P† |
|----------------|------------|----|----|----|---|---|----|
| rs11024600     | TWB        | 11.9 (7.7-22.2)* (733) | 7.9 (4.8-13.3) (1062) | 2.6 (1.4-5.0) (402) | 3.65×10^-13 | -0.351 | 3.84×10^-14 |
|                | CAD        | 18.9 (9.8-63.5) (155) | 9.7 (5.4-21.8) (241) | 4.5 (2.7-9.4) (82) | 4.54×10^-17 | -0.342 | 5.18×10^-12 |
| rs7112278      | TWB        | 5.6 (2.8-9.1) (1024) | 9.8 (6.4-17.7) (969) | 15.3 (8.8-28.4) (204) | 2.10×10^-9 | 0.287 | 6.58×10^-78 |
|                | CAD        | 8.7 (4.2-21.6) (227) | 12.3 (6.7-33.7) (215) | 21.2 (12.3-177.2) (37) | 4.44×10^-6 | 0.225 | 4.01×10^-5 |
| SAA-WGRS       |             | 0.732 | 1.53×10^-17 | 0.756 | 3.17×10^-13 |

*Median (IQR) (number of participants), †P: Unadjusted, Kruskal–Wallis test, ††P: Adjusted for sex, age, BMI, current smoking status. BMI: Body mass index, IQR: Interquartile range, M: Major allele, m: Minor allele, TWB: Taiwan Biobank, CAD: Coronary artery disease, WGRS: Weighted genetic risk score, SAA: Serum amyloid A

Associations between serum amyloid A levels and inflammatory marker levels and leukocyte counts in patients with coronary artery disease in Taiwan

When the SAA levels and inflammatory marker levels, such as CRP, sICAM1, sE-selectin, and MMP9 levels, were compared in patients with CAD, a positive association between levels of SAA and all four inflammatory markers was noted [Figure 3a-d]. This is in contrast to our previously reported health examination participants in Taiwanese in which, when compared to the comparison of similar biomarkers, only CRP presented evidence of significant association. These results may suggest a more crucial role of inflammation in determining SAA levels in patients with CAD. By contrast, a significant association between leukocyte counts and SAA levels was noted in CAD patients, similar to TWB participants [Figure 3e].

Stepwise linear regression analysis of serum amyloid A levels

For the TWB population, age, sex, BMI, and HDL-C and ALT levels and leukocyte counts were independently associated with SAA1 levels in multivariate analysis [Table 4]. By using stepwise regression with adjustments, two SAA SNPs (rs11024600 and rs7112278) remained independently associated with SAA1 levels and contributed to 23.78% and 3.75% of the variation, respectively [Table 4]. For the patients with CAD, rs11024600 and rs7112278 genotypes, leukocyte...
counts, and CRP and sICAM1 levels were independently associated with SAA levels. A combination of SAA1 gene variants contributed to 27.53% and 8.07% variation in SAA levels in TWB participants and patients with CAD, respectively, revealing a stronger influence of these two variants in TWB participants compared to CAD patients.

Circulating serum amyloid A levels, serum amyloid A1 genotypes, and long-term prognosis in patients with coronary artery disease

In patients with CAD, the follow-up time was 1022 ± 320 days (minimum: 5 days; maximum: 1460 days). During the follow-up period, 20 patients developed heart failure and were hospitalized, 10 patients had stroke, three patients had myocardial infarction, and 27 patients died. Due to overlapping clinical manifestations, 54 patients reached the secondary endpoint. The comparison between patients with CAD with or without secondary endpoints is shown in Table 2. Female sex, mean age, CRP and sICAM1 levels, leukocyte counts, diabetes frequency, multivessel diseases, prior congestive heart failure, and acute coronary syndrome were significantly higher and hematocrit and eGFR were significantly lower in patients who reached the
secondary endpoint than in patients that did not. Patients who reached the secondary endpoint had significantly higher baseline SAA levels than those who did not reach the secondary endpoint [median (IQR) = 21.13 (9.02–287.86) vs. 10.21 (5.27–24.79) μg/mL, P < 0.001]. From the ROC curve analysis and the Youden index, the best prognostic cutoff value was 51.04 μg/mL per SAA level [Supplementary Figure 3]. Kaplan–Meier survival analysis revealed that a high SAA level was a strong predictor of mortality and reaching the secondary endpoint [Figure 4; P = 2.0 × 10⁻⁶ and 2.7 × 10⁻⁸, respectively]. We also tested whether SAA1 SNPs were associated with clinical outcomes of CAD, and no significant difference between SAA1 genotypes/ WGRSs and long-term outcome in patients with CAD was noted [Figure 4]. Cox regression analysis indicated that higher circulating SAA levels, but not high SAA1 WGRS, were independent predictors of reaching the primary and secondary endpoints after using different adjustment models [Table 5].

**Discussion**

This investigation used TWB participants for a GWAS and revealed that the SAA1 gene is the only locus for SAA levels with a genome-wide significant association in a Taiwanese population. Compared with the healthy general population, genetic factors possibly play a less important role, whereas inflammatory biomarker levels appeared to be more crucial to the elevation of SAA levels in patients with CAD. Furthermore, our data revealed that circulating SAA levels predicted the long-term outcomes of angiographically confirmed CAD, whereas SAA1 genetic variants are not strong enough to predict the CAD prognosis.
Table 5: Serum amyloid A level and serum amyloid A-weighted genetic risk scores as a predictor of primary and secondary endpoints in Cox regression analysis in patients with coronary artery disease

|                | Model 1       | Model 2       | Model 3       |
|----------------|---------------|---------------|---------------|
| Primary endpoint |               |               |               |
| SAA level (high vs. low) | 5.183 (2.436-11.029) | 4.388 (1.989-9.684) | 3.399 (1.517-7.616) |
| HR (95% CI)    | 0.000019      | 0.00025       | 0.003         |
| WGRS_SAA1 (high vs. low) | 2.1464 (0.584-10.403) | 2.346 (0.547-10.066) | 2.720 (0.617-11.991) |
| HR (95% CI)    | 0.2198        | 0.2513        | 0.1861        |
| Secondary endpoint |               |               |               |
| SAA level (high vs. low) | 4.083 (2.385-6.988) | 3.472 (2.003-6.021) | 2.721 (1.548-4.781) |
| HR (95% CI)    | 2.885×10⁻⁷   | 0.000009      | 0.001         |
| WGRS_SAA1 (high vs. low) | 2.446 (0.883-6.777) | 2.385 (0.854-6.660) | 2.173 (0.772-6.116) |
| HR (95% CI)    | 0.0855        | 0.0971        | 0.1418        |

Table 4: Stepwise multivariate linear regression analysis of serum amyloid A levels in two study populations

|                          | TWB participants | CAD patients |
|--------------------------|-----------------|--------------|
|                          | β     | R² | P   | β     | R²  | P   |
| Age (years)              | 0.0050 | 0.0103 | 2.56×10⁻⁰⁰ | -   | -   | -   |
| Sex (female vs. male)    | 0.1124 | 0.0082 | 1.63×10⁻⁹  | -   | -   | -   |
| BMI (kg/m²)              | 0.0271 | 0.0369 | 5.30×10⁻²² | -0.0168 | 0.0065 | 0.0034 |
| Current smoking status   | -     | -   | -   | -     | -   | -   |
| SAA1 rs11024600 genotypes | -0.2932 | 0.2378 | 1.71×10⁻¹⁰¹ | 0.0379 | 0.0688 | 1.51×10⁻¹⁴ |
| SAA1 rs7112278 genotypes | 0.1696 | 0.0375 | 2.77×10⁻²² | -0.0176 | 0.0119 | 0.0001 |
| Leukocyte counts (10⁹/mL) | 0.0473 | 0.0140 | 2.84×10⁻⁶   | 0.0631 | 0.0248 | 7.19×10⁻⁸  |
| HDL cholesterol level    | 0.3561 | 0.0139 | 4.85×10⁻⁶   | -     | -   | -   |
| ALT level                | 0.0018 | 0.0074 | 2.98×10⁻⁷   | 0.8333 | 0.4872 | 9.72×10⁻₅³ |
| CRP (mg/L)               | -     | -   | -   | 0.8774 | 0.0132 | 1.26×10⁻³  |
| sICAM1 (ng/mL)           | -     | -   | -   | -     | -   | -   |
| sE-selectin (ng/mL)      | -     | -   | -   | -     | -   | -   |
| MMP9 (ng/mL)             | -     | -   | -   | -     | -   | -   |

Table: Association between serum amyloid A levels and baseline characteristics in Taiwan Biobank participants

We previously reported that SAA levels in a health examination population exhibited a trend but no significant association between waist-to-hip ratio, eGFR, fasting plasma glucose, HDL-C level, and urinary albumin-to-creatinine ratio [23]. In this study, with a larger population for analysis, we noted associations of SAA levels with multiple metabolic phenotypes and biochemical and hematological parameters, and at multivariate analysis, age, sex, BMI, and HDL-C and ALT levels and leukocyte counts showed an independent association with SAA level. Acute-phase SAA mRNA level was highly and selectively expressed in human adipocytes [28]. Similar to our study, a strong association between BMI and SAA levels was found in a meta-analysis [29]. By contrast, the associations between SAA level and HDL-C level are more controversial. Zhou et al. [16], in a meta-analysis in patients with CAD, showed that high levels of SAA are negatively associated with HDL-C levels. In a large population with 2280 participants in the Cardiovascular Risk in Young Finns Study, the negative association of SAA levels with HDL-C levels occurred only in men, and the association disappeared after further adjustment of BMI [30]. We have tested the association between SAA level and HDL-C levels according to sex in TWB participants and found a positive association predominantly in women [Supplementary Table 4]. Thus, further larger studies in different ethnic populations may help to elucidate the correlation between SAA and HDL-C levels in diverse disease status. In the analysis of 278 patients with different liver diseases, Yuan et al. [31] noted increased SAA levels in patients with active chronic hepatitis B, nonalcoholic steatohepatitis, drug-induced liver injury, autoimmune liver disease, and pyogenic liver abscess. This is compatible with...
our relatively healthy TWB participants, in which SAA level was positively associated with elevated ALT level.

**Comparison between genome-wide association studies for genetic determinants of serum amyloid A levels**

Marzi *et al.* [22] reported that the *SAA1* locus was the most crucial genetic determinant of the circulating SAA level. The *LEPR* gene at 1p31 was the second region associated with the SAA level, as determined in GWAS in European populations. By combining these two regions, the SNPs explained 11.68% of the variance, which comprised 19.76% of the total estimated heritability of 59% [22]. In our GWAS analysis, the *SAA1* locus was the only gene region associated with SAA level. The lead SNP, *SAA1* rs11024600, explained nearly 24% of the variance in SAA levels, and when combined with the *SAA1* SNP rs7112278, genetic factors could explain 27.53% of the variance in SAA levels. These results indicated the crucial role of *SAA1* gene variants on SAA levels in diverse ethnic populations. *LEPR* SNPs were previously associated with CRP, fibrinogen levels, and leukocyte counts [32-35] and recently for SAA levels. However, our data did not support the association of *LEPR* polymorphisms with SAA levels in the Taiwanese participants. Determining whether *LEPR* polymorphisms are crucial for SAA levels in Taiwanese or other Asian populations may require studies with a larger sample size.

**Association between serum amyloid A levels and proinflammatory marker levels in different populations**

Chronic inflammation is a crucial component of the development and progression of atherosclerosis [36-38]. Elevated inflammatory biomarkers have been detected in many studies involving patients with atherosclerotic cardiovascular disease [39,40]. We found a positive association between SAA levels and leukocyte counts in both TWB participants and patients with CAD. We also tested the association between various inflammatory marker levels and discovered that SAA and inflammatory marker levels in angiographically confirmed CAD patients and found a significant association between SAA level and CRP, sICAM1, sE-selectin, and MMP9 levels. This is in compatible with those reported by Zhou *et al.* [16] which showed positive association between SAA level and CRP, fibrinogen and interleukin 6 levels. These results suggested that inflammatory pathways appeared to be more crucial to the elevation of SAA levels in patients with CAD.

**Association between serum amyloid A1 single-nucleotide polymorphisms and serum amyloid A levels in healthy participants and patients with coronary artery disease**

In this study, we confirmed the significant association of *SAA1* rs11024600 and rs7112278 genotypes with SAA levels in patients with CAD. The rs4638289 genotypes, previously reported to be strongly associated with SAA levels, had nearly complete linkage disequilibrium with the rs7112278 genotypes. The *SAA1* genotypes contributed to 8.07% of the variation in SAA levels in patients with CAD, lower than that of healthy participants. Elevated SAA levels are strongly indicative of the risk and severity of atherosclerotic lesions and cardiovascular disease events [17-20]. These results suggested that as atherosclerosis progresses, the inflammatory effect but not genetic factors contribute to an increasingly significant portion of SAA levels in patients with CAD. However, even with elevated SAA levels, the WGRS of *SAA1* genotypes remained significantly associated with SAA levels, which suggested the crucial role of the genetic components of SAA levels in patients with CAD.

**Circulating serum amyloid A levels but not serum amyloid A1 genetic variants predict long-term outcomes of coronary artery disease**

Circulating SAA levels may be a powerful predictor of all-cause mortality and adverse cardiovascular outcomes in patients with CAD, particularly in patients with acute coronary syndrome or acute myocardial infarction [41-44]. However, during a median follow-up period of 11.8 years, SAA level was found not independently associated with mortality in patients with subclinical carotid atherosclerosis [45]. We extended the above observations to patients with angiographically documented CAD, showing SAA levels predicted the long-term outcomes of CAD. By contrast, the influence of baseline SAA levels due only to *SAA1* polymorphisms may not be large enough to reduce mortality risk in patients with CAD. The decreasing power of genetic factors for determining biomarker levels in patients with CAD than in the health examination sample or the general population was identified in most of our previous studies [24,25,27].

**Limitations**

The CAD population and number of patients who reached outcomes were small, and larger and longer follow-up studies may need to verify our results. The study participants were ethnically Chinese; hence, caution should be exercised when extrapolating our results to other ethnic groups.

**Conclusion**

Our results confirmed that SAA levels but not *SAA1* genotypes are a strong predictor of long-term outcomes in patients with CAD. By using a GWAS, we obtained two *SAA1* SNPs, rs11024600 and rs7112278, which in combination contributed to nearly half of the heritability predicted by previous twin studies [21]. However, in patients with CAD, the two SNPs contributed to less effect on SAA levels. Larger-scale prospective studies in the future can help determine the definite role of *SAA1* genotypes as a predictor of the long-term outcomes of various atherosclerotic cardiovascular diseases.

**Acknowledgments**

We greatly appreciate technical support from the Core Laboratory of the Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, and expert statistical analysis assistance from Tsung-Han Hsieh.

**Financial support and sponsorship**

This study was supported by grants from the Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation (TCRD-TPE-MOST-108-05, TCRD-TPE-109-RT-1) and Buddhist Tzu Chi Medical Foundation Academic Advancement (TCMF-EP 108-05), grants from the Ministry of Science and
Technology (MOST 108-2314-B-303 -026 -MY3) to Y. L. Ko, and the Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation (TCRD-TPE-107-53) to K. H. Yeh.

Conflicts of interest

Dr. Yu-Lin Ko, an editorial board members at Tzu Chi Medical Journal, had no role in the peer review process of or decision to publish this article. The other authors declared no conflicts of interest in writing this paper.

REFERENCES

1. Uhlar CM, Whitehead AS. Serum amyloid A, the major vertebrate acute-phase reactant. Eur J Biochem 1999;265:501-23.
2. Casl MT, Surina B, Glojnarić-Spasić I, Pape E, Jagarinec N, Kranjcević S. Serum amyloid A protein in inflammatory atherosclerotic lesions and cultured vascular cells: Implications for serum amyloid A function. Proe Natl Acad Sci U S A 1994;91:3186-90.
3. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Engl J Med 1999;340:448-54.
4. Meek RL, Urieli-Shoval S, Benditt EP. Expression of apolipoprotein serum amyloid A mRNA in human atherosclerotic lesions and cultured vascular cells: The role of serum amyloid A protein. Proc Natl Acad Sci U S A 1994;91:3186-90.
5. Rockl H, Loose LD, Bartle LM, Sipe JD. Synergism of interleukin 1 and interleukin 6 induces serum amyloid A production while depressing fibrinogen: A quantitative analysis. J Rheumatol 1994;21:400-5.
6. King VL, Thompson J, Tannock LR. Serum amyloid A in atherosclerosis. Curr Opin Lipidol 2011;22:302-7.
7. Kisilevsky R, Tam SP. Acute phase serum amyloid A, cholesterol metabolism, and cardiovascular disease. Pediatr Pathol Mol Med 2002;21:291-305.
8. Han CY, Tang C, Guevara ME, Wei H, Wietecha T, Shao B, et al. Serum amyloid A impairs the anti-inflammatory properties of HDL. J Clin Invest 2016;126:266-81.
9. Wilson PG, Thompson JC, Shridas P, McNamara PJ, de Beer MC, de Beer FC, et al. Serum amyloid A is an exchangeable apolipoprotein. Arterioscler Thromb Vasc Biol 2018;38:1890-900.
10. Shridas P, Tannock LR. Role of serum amyloid A in atherosclerosis. Curr Opin Lipidol 2019;30:320-5.
11. Dong Z, Wu T, Qin W, An C, Wang Z, Zhang M, et al. Serum amyloid A directly accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice. Mol Med 2011;17:1357-64.
12. Thompson JC, Jayne C, Thompson J, Wilson PG, Yoder MH, Webb N, et al. Brief elevation of serum amyloid A is sufficient to increase atherosclerosis. J Lipid Res 2015;56:286-93.
13. Song C, Hsu K, Yamen E, Yan W, Fock J, Witting PK, et al. Serum amyloid A induction of cytokines in monocytes/macrophages and lymphocytes. Atherosclerosis 2009;207:374-83.
14. Sack GH Jr. Serum amyloid A (SAA) proteins. Subcell Biochem 2020;94:421-36.
15. Soric Hosman I, Kos I, Lamot L. Serum amyloid A in inflammatory rheumatic diseases: A comprehensive review of a renowned biomarker. Front Immunol 2020;11:631299.
16. Zhou J, Lu Y, Wang S, Chen K. Association between serum amyloid A levels and coronary heart disease: A systematic review and meta-analysis of 26 studies. Inflamm Res 2020;69:331-45.
17. Danesh J, Wheeler JG, Hirschfeld GM, Eda S, Eiriksdottir G, Rumley A, et al. C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. N Engl J Med 2004;350:1387-97.
18. Fyfe AJ, Rothenberg LS, DeBeer FC, Cantor RM, Rotter JL, Lewis AJ. Association between serum amyloid A proteins and coronary artery disease: Evidence from two distinct arteriosclerotic processes. Circulation 1997;96:2914-9.
19. Schillinger M, Exner M, Mlekusch W, Sabeti S, Amighi J, Nikowitsch R, et al. Inflammation and carotid artery – risk for atherosclerosis study (ICARAS). Circulation 2005;111:2203-9.
20. Johnson BD, Kip KE, Marroquin OC, Ridker PM, Kelsey SF, Shaw LJ, et al. Serum amyloid A as a predictor of coronary artery disease and cardiovascular outcome in women: The National Heart, Lung, and Blood Institute-Sponsored Women’s Ischemia Syndrome Evaluation (WISE). Circulation 2004;109:726-32.
21. MacGregor AJ, Gallimore JR, Spector TD, Pepys MB. Genetic effects on baseline values of C-reactive protein and serum amyloid a protein: A comparison of monozygotic and dizygotic twins. Clin Chem 2004;50:130-4.
22. Marzi C, Albrecht E, Hysi PG, Lagou V, Waldenberger M, Tönjes A, et al. Genome-wide association study identifies two novel regions at 11p15.5-p13 and 1p31 with major impact on acute-phase serum amyloid A. PLoS Genet 2010;6:e1001213.
23. Ko YL, Huang T, Seng MS, Chou HH. CRP and SAA1 haplotypes are associated with both C-reactive protein and serum amyloid A levels: Role of suppression effects. Mediators Inflamm 2016;2016:5830361.
24. Hsu LA, Wu S, Jiang JJ, Chiang FT, Teng MS, Lin JF, et al. Growth differentiation factor 15 May predict mortality of coronary and peripheral artery diseases and correlate with their risk factors. Mediators Inflamm 2017;2017:938401.
25. Lin JF, Wu S, Jiang JJ, Chiang FT, Hsu LA, Teng MS, et al. IL1R1 single nucleotide polymorphism predicts sST2 level and mortality in coronary and peripheral arterial disease. Atherosclerosis 2017;257:71-7.
26. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, et al. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 2009;53:982-92.
27. Suri K, Hsu LA, Jiang JH, Wang MS, Tseng IS, et al. Circulating ceremherin levels, but not the RARRES2 polymorphisms, predict the long-term outcome of angiographically confirmed coronary artery disease. Int J Mol Sci 2019;20:1174.
28. Yang RZ, Lee MJ, Hu H, Pollin TI, Ryan AS, Nicklas BJ, et al. Acute-phase serum amyloid A: An inflammatory adipokine and potential link between obesity and its metabolic complications. PLoS Med 2006;3:e287.
29. Zhao Y, He X, Shi X, Huang C, Liu J, Zhou S, et al. Association between serum amyloid A and obesity: a meta-analysis and systematic review. Inflamm Res 2010;59:323-34.
30. Jylhävä J, Haarala A, Eklund C, Pertovaara M, Kähönen M, Hutri-Kähönen N, et al. Serum amyloid A is independently associated with metabolic risk factors but not with early atherosclerosis: The cardiovascular risk in young Finns study. J Intern Med 2009;266:286-95.
31. Yuan ZY, Zhang XX, Wu YJ, Zeng ZP, She WM, Chen SY, et al. Serum amyloid A levels in patients with liver diseases. World J Gastroenterol 2015;21:6440-50.
36. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. Circulation 2002;105:1135-43.
37. Ross R. Atherosclerosis – An inflammatory disease. N Engl J Med 1999;340:115-26.
38. Hansson GK. Immune mechanisms in atherosclerosis. Arterioscler Thromb Vasc Biol 2001;21:1876-90.
39. Lind L. Circulating markers of inflammation and atherosclerosis. Atherosclerosis 2003;169:203-14.
40. Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000;342:836-43.
41. Katayama T, Nakashima H, Takagi C, Honda Y, Suzuki S, Iwasaki Y, et al. Prognostic value of serum amyloid A protein in patients with acute myocardial infarction. Cire J 2005;69:1186-91.
42. Morrow DA, Rifai N, Antman EM, Weiner DL, McCabe CH, Cannon CP, et al. Serum amyloid A predicts early mortality in acute coronary syndromes: A TIMI 11A substudy. J Am Coll Cardiol 2000;35:358-62.
43. Song C, Shen Y, Yamam E, Hsu K, Yan W, Witting PK, et al. Serum amyloid A may potentiate prothrombotic and proinflammatory events in acute coronary syndromes. Atherosclerosis 2009;202:596-604.
44. Zamani P, Schwartz GG, Olsson AG, Rifai N, Bao W, Libby P, et al. Inflammatory biomarkers, death, and recurrent nonfatal coronary events after an acute coronary syndrome in the MIRACL study. J Am Heart Assoc 2013;2:e003103.
45. Mayer FJ, Binder CJ, Krychtiuk KA, Schillinger M, Minar E, Hoke M. The prognostic value of serum amyloid A for long-term mortality among patients with subclinical carotid atherosclerosis. Eur J Clin Invest 2019;49:e13095.
**Supplementary Method**

**Genome-Wide Association Study Analysis**

In this investigation, all the samples enrolled for the analysis had a call rate of ≥97%. SNP quality control (QC) was set as follows: An SNP call rate of <3%, a minor allele frequency of <0.05, and a violation of Hardy–Weinberg equilibrium ($P < 10^{-6}$); these were excluded from subsequent analyses. After QC, a total of 614,821 SNPs were enrolled for the GWAS analysis.

**Supplementary Figure 1:** Linkage disequilibrium of SAA1 single-nucleotide polymorphisms

**Supplementary Figure 2:** Regional plot of LEPR gene region in the genome-wide association study for serum amyloid A levels. None of the single-nucleotide polymorphisms within 20 kb of the LEPR gene region reach $P < 0.001$.

**Supplementary Figure 3:** Receiver operating characteristic curves of serum amyloid A levels for secondary outcomes of patients with coronary artery disease.
Supplementary Table 2: Serum amyloid A levels according to the cardiovascular risk factors and lifestyle in the Taiwan Biobank population

|              | n  | SAA levels | P1     | P2     | Adjusted P2 |
|--------------|----|------------|--------|--------|-------------|
| Sex          |    |            |        |        |             |
| Male         | 987| 7.61 (3.84-13.49) | 0.004  | 1.40×10⁻⁴ | 7.00×10⁻⁵  |
| Female       | 1212| 8.34 (4.87-15.22) |         |        |             |
| Obesity      |    |            |        |        |             |
| No           | 1362| 7.24 (3.80-12.50) | 1.59×10⁻¹³ | 1.45×10⁻¹³ | 7.25×10⁻¹⁴ |
| Yes          | 837 | 9.37 (5.80-18.31) |         |        |             |
| Hypertension |    |            |        |        |             |
| No           | 1857| 7.81 (4.26-13.95) | 0.046  | 0.161  | 0.805       |
| Yes          | 342 | 8.72 (4.98-17.16) |         |        |             |
| Diabetes mellitus |  |            |        |        |             |
| No           | 2069| 8.00 (4.31-14.24) | 0.295  | 0.444  | 2.220       |
| Yes          | 130 | 8.25 (5.14-16.00) |         |        |             |
| Current smoker| |            |        |        |             |
| No           | 1803| 8.15 (4.62-14.36) | 0.023  | 0.339  | 1.965       |
| Yes          | 396 | 7.20 (3.68-13.76) |         |        |             |

P1 value: Unadjusted (Independent t-test), P2 value: Adjusted for age and sex, (Linear regression), Adjusted P2 value, Bonferroni correction with (n=5) Sex: Adjusted for age, BMI and current smoking status, Current smoker: Adjusted for age, sex, and BMI, Obesity: Adjusted for age, sex, and current smoker. SAA: Serum amyloid A, BMI: Body mass index.
### Supplementary Table 3: Serum amyloid A single-nucleotide polymorphisms with genome-wide significance in our genome-wide association study

| rs number     | Position   | Gene               | Genotypes | MAF     | β       | SE     | P         |
|---------------|------------|--------------------|-----------|---------|---------|--------|-----------|
| rs12282742    | 18265799   | SAA2, Intron variant | C/T       | 0.066   | 0.236   | 0.029 | 4.24×10^{-10} |
| rs10832911    | 18275661   | Intergenic         | G/A       | 0.437   | 0.204   | 0.014 | 1.19×10^{-44} |
| rs57322649    | 18278136   | Intergenic         | C/T       | 0.430   | 0.315   | 0.013 | 6.29×10^{-114} |
| rs1993373     | 18280635   | SAA1               | A/C       | 0.203   | 0.286   | 0.015 | 3.24×10^{-77}  |
| rs7112278     | 18281916   | SAA1               | T/C       | 0.304   | 0.287   | 0.015 | 6.58×10^{-78}  |
| rs11603089    | 18282051   | SAA1               | A/G       | 0.208   | 0.071   | 0.018 | 8.70×10^{-5}   |
| rs11024595    | 18287798   | SAA1               | C/T       | 0.416   | 0.321   | 0.013 | 1.01×10^{-118} |
| rs11024600    | 18295810   | Intergenic         | T/C       | 0.446   | 0.351   | 0.013 | 3.84×10^{-145} |
| rs11024603    | 18306399   | HPS5, Intron variant | G/A       | 0.378   | 0.294   | 0.014 | 7.99×10^{-92}  |
| rs7128146     | 18319279   | HPS5, Intron variant | G/T       | 0.108   | 0.163   | 0.024 | 1.61×10^{-11}  |
| rs2403254     | 18325146   | HPS5, Intron variant | T/C       | 0.449   | 0.267   | 0.014 | 1.38×10^{-79}  |
| rs7113249     | 18331628   | HPS5, Intron variant | A/G       | 0.108   | 0.164   | 0.024 | 1.40×10^{-11}  |
| rs6088939     | 18352433   | GTF2H1, Intron variant | G/A       | 0.425   | 0.279   | 0.014 | 1.11×10^{-44}  |
| rs4150599     | 18362457   | GTF2H1, Intron variant | A/G       | 0.317   | 0.244   | 0.015 | 1.24×10^{-57}  |
| rs4757645     | 18393845   | Intergenic         | G/C       | 0.362   | 0.209   | 0.014 | 3.95×10^{-45}  |
| rs1881717     | 18472133   | LDHC, Intron variant | G/A       | 0.344   | 0.090   | 0.015 | 2.52×10^{-9}   |

*Transcriptional factor binding site. P value: Adjusted for sex, age, BMI, current smoking status, BMI: Body mass index, MAF: Minor allele frequency, SE: Standard error, SAA: Serum amyloid A

### Supplementary Table 4: Association between serum amyloid A level and high-density lipoprotein cholesterol level according to sex

| TWB male | β       | R²       | P         | TWB female | β       | R²       | P         |
|----------|---------|----------|-----------|------------|---------|----------|-----------|
| Age (years) | 0.0033  | 0.0039   | 6.63E-03  | 0.007     | 0.0168  | 1.26E-07 |
| BMI (kg/m²) | 0.0229  | 0.0384   | 3.54E-08  | 0.0279    | 0.0309  | 4.31E-15 |
| Current smoking status | -0.0553 | 0.0018   | 0.0499    | -         | -       | -         |
| SAA1 rs11024600 genotypes | -0.3060 | 0.2562   | 6.86E-49  | -0.2838   | 0.2265  | 3.36E-55 |
| SAA1 rs7112278 genotypes | 0.1896  | 0.0487   | 9.38E-18  | 0.1539    | 0.0488  | 2.45E-16 |
| Leukocyte counts (10³/μL) | 0.0545  | 0.0193   | 9.09E-10  | 0.0409    | 0.0183  | 8.83E-08 |
| HDL cholesterol level | -       | -        | -         | 0.7439    | 0.0144  | 6.39E-10 |
| ALT level | 0.0009  | 0.0020   | 0.0444    | 0.0031    | 0.0101  | 2.02E-07 |

BMI: Body mass index, HDL: High-density lipoprotein, SAA: Serum amyloid A, TWB: Taiwan biobank, ALT: Alanine aminotransferase