Original Article

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

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

[a]Cardiovascular Center and Division of Cardiology, Department of Internal Medicine, Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, New Taipei, Taiwan, [b]School of Medicine, Tzu Chi University, Hualien, Taiwan, [c]The First Cardiovascular Division, Department of Internal Medicine, Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Taoyuan, Taiwan, [d]Cardiovascular Center and Division of Cardiology, Department of Internal Medicine, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan, [e]Division of Cardiology, Department of Internal Medicine, National Taiwan University Hospital and Fu-Jen Catholic University Hospital, Fu-Jen Catholic University, Taipei, Taiwan, [f]Department of Research, Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, New Taipei, Taiwan, [g]Department of Life Science, Chinese Culture University, Taipei, Taiwan, [h]School of Post-Baccalaureate Chinese Medicine, Tzu Chi University, Hualien, Taiwan

ABSTRACT

Objectives: Circulating serum amyloid A (SAA) levels are strongly associated with atherosclerotic cardiovascular disease risk and severity. The association between *SAA1* genetic 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 ($P = 3.84 \times 10^{-145}$ 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

Supplementary materials available online
Access this article online
Quick Response Code:
Website: www.tcmjmed.com
DOI: 10.4103/tcmj.tcmj_219_21

*Address for correspondence: Dr. Yu-Lin Ko,
Division of Cardiology, Department of Internal Medicine, Taipei Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, 289, Jianguo Road, Xindian District, New Taipei, Taiwan.
E-mail: yulinkotw@yahoo.com.tw

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 pathophysiological 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, 11p15.5-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: $175 \times$ serum creatinine$^{-1.154} \times$ age$^{-0.203}$ ($\times$ 0.742 for female patients) [26]. The levels of inflammatory markers,
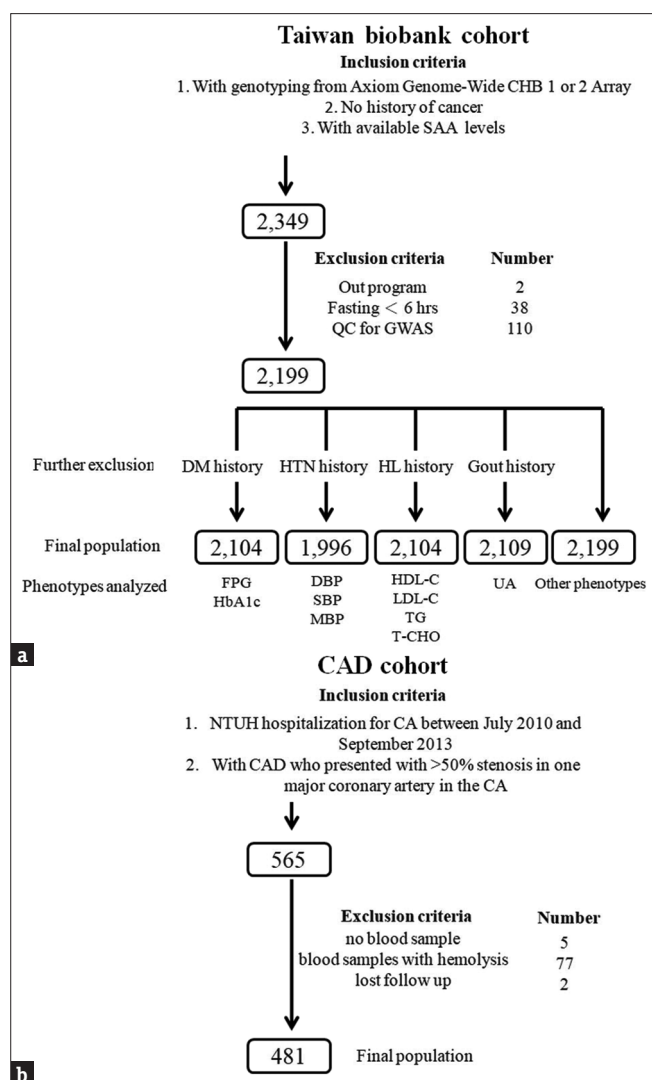
Figure 1: Study flowchart of inclusion and exclusion criteria used to screen Taiwan Biobank project participants (a) and patients with coronary artery disease (b). Other phenotypes include age; body mass index; waist circumference; waist–hip ratio; aspartate aminotransferase, alanine aminotransferase, γ-glutamyl transferase, and serum creatinine levels; estimated glomerular filtration rate; serum albumin; total bilirubin; hemoglobin; hematocrit; red blood cell, leukocyte, and platelet counts; and blood urea nitrogen, albuminuria, microalbuminuria, and metabolic syndrome. QC: Quality control, HL: Hyperlipidemia, HTN: Hypertension, DM: Diabetes mellitus, FPG: Fasting plasma glucose, HbA1C: Hemoglobin A1C, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MBP: Mean blood pressure, LDL-C: Low-density lipoprotein cholesterol, HDL-C: High-density lipoprotein cholesterol, TG: Triglyceride, T-CHO: Total cholesterol, UA: Uric acid

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 was conducted by adding the most strongly associated SNP at a locus into the regression model as a covariate and by testing the residual association with all remaining SNPs.

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, and BMI. 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, and leukocyte counts.

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 $-\log_{10} P$ 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	$2.63×10^{-4}$	0.0717	$7.71×10^{-4}$	0.0185
Waist circumference (cm)	2199	83.0 (77.0-89.8)	0.153	$6.30×10^{-13}$	0.0440	0.0391	0.9387
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	$4.41×10^{-16}$	0.1922	$1.02×10^{-19}$	$2.44×10^{-18}$
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 cholesterol#	2104	192.0 (170.0-217.0)	0.094	$1.40×10^{-5}$	0.0640	$3.34×10^{-3}$	0.0802
HDL-cholesterol#	2104	54.0 (45.0-64.0)	0.044	0.043	0.0862	$7.60×10^{-5}$	$1.82×10^{-3}$
LDL-cholesterol#	2104	120.0 (99.0-143.0)	0.072	0.001	0.0406	0.0627	0.9999
Triglyceride#	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/1.75 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	$1.60×10^{-5}$	$3.84×10^{-4}$
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 (10³/μL)	2199	5.9 (5.0-7.0)	0.135	$2.05×10^{-10}$	0.1173	$3.61×10^{-8}$	$8.65×10^{-7}$
Hemoglobin (g/dL)	2199	14.0 (13.0-15.1)	0.025	0.241	0.0685	0.0013	0.0315
Platelet count (10³/μL)	2199	235.0 (201.0-273.0)	0.029	0.171	0.0079	0.7100	0.9999

$^†P$ value: Unadjusted, $^{††}P$ value: Adjusted for sex, age, BMI, smoking status, §Adjusted P value, adjusted for sex, age, BMI, smoking status with Bonferroni correction (n=24), *Were analyzed with the exclusion of participants with previous history of hypertension, **Were analyzed with the exclusion of participants with previous history of diabetes mellitus, ***Were analyzed with the exclusion of participants with previous history of gout, #Were 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 ($P = 3.84 × 10^{-145}$), 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 S*AA1* 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

	Total (481)	Without secondary end point (427)	With secondary end point (54)	P
Baseline characteristics				
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^{-6}
Body mass index (kg/m^2)	25.6 (23.5-28.3)	25.7 (23.5-28.4)	25.1 (22.7-27.5)	0.1068
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/MI	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 (1.0-2.1)	8.95×10^{-6}
eGFR (mL/min/1.75m^2)	67.8 (54.3-80.7)	68.5 (55.6-81.7)	51.7 (29.7-68.4)	1.29×10^{-6}
Blood cell counts				
Leukocyte counts (10^3/μL)	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^{-6}
Platelet counts (10^3/μL)	205.0 (176.0-241.5)	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^{-5}
SAA (μg/mL)	11.2 (5.4-27.6)	10.2 (5.3-24.8)	21.1 (9.0-287.9)	5.00×10^{-5}
sICAM1 (ng/mL)	126.4 (107.6-149.4)	123.9 (106.4-144.2)	150.3 (123.0-178.8)	3.27×10^{-6}
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	Mm	mm	$P^{\dagger}$	β	$P^{\dagger\dagger}$
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^{-132}	−0.351	3.84×10^{-145}
	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^{-80}	0.287	6.58×10^{-78}
	CAD	8.7 (4.2-21.6) (227)	12.3 (6.7-33.7) (215)	21.2 (12.3-117.2) (37)	4.44×10^{-6}	0.225	4.01×10^{-5}
SAA-WGRS	TWB					0.732	1.53×10^{-170}
	CAD					0.756	3.17×10^{-12}

*Median (IQR) (number of participants), $^{\dagger}P$: Unadjusted, Kruskal–Wallis test, $^{\dagger\dagger}P$: 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 *SAA1* 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
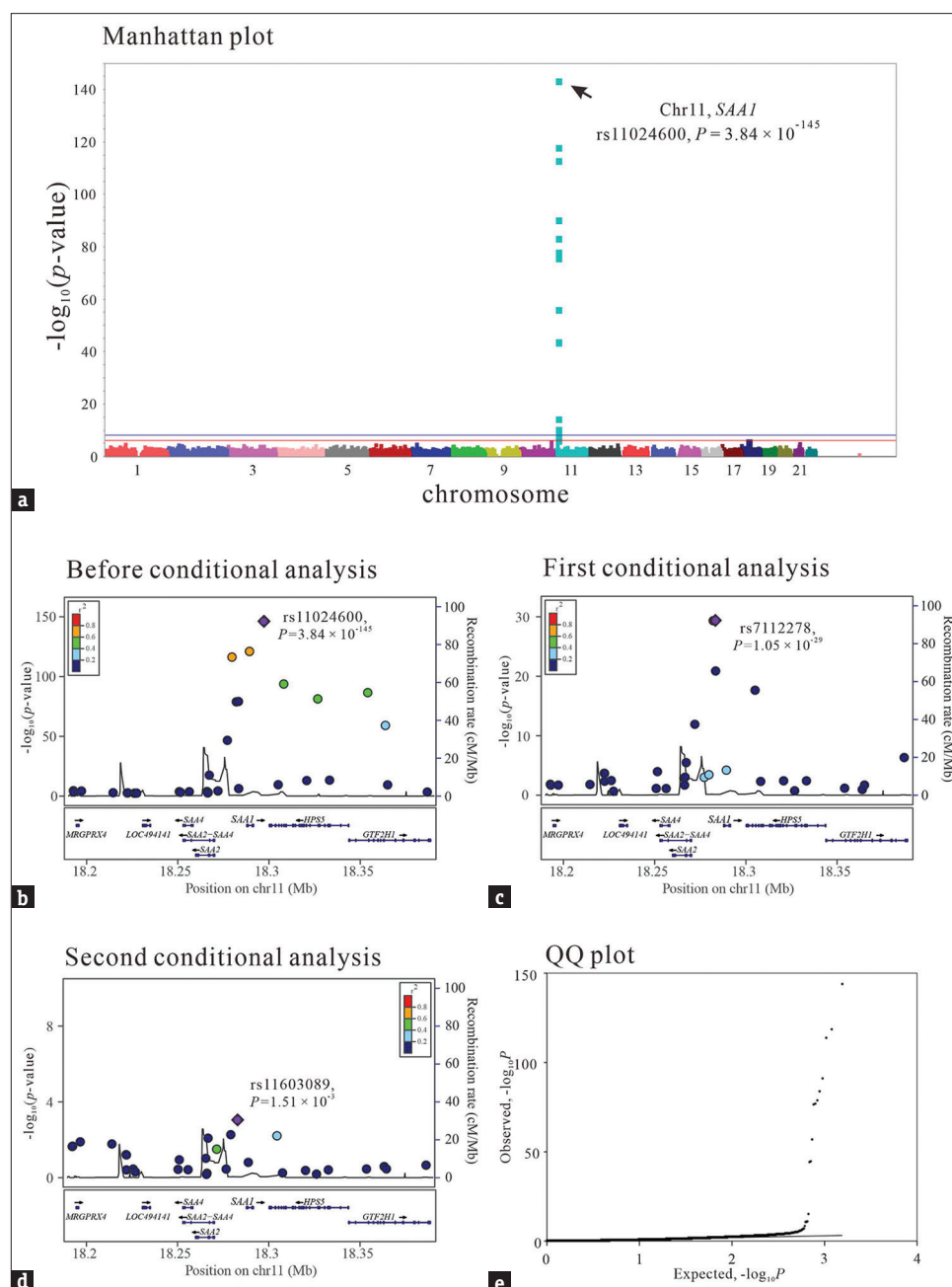
Figure 2: Manhattan plot (a) and regional association plots (b-d) of genome-wide association study for SAA levels without (a and b) or with (c and d) conditional analysis of the *SAA1* polymorphisms. The data revealed two independent *SAA1* polymorphisms with genome-wide significance association for SAA levels in Taiwanese. The quantile-quantile plot points out the observed significant associations beyond those expected by chance (e). *P* value adjusted for adjusted for age, sex, current smoking status, and body mass index

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
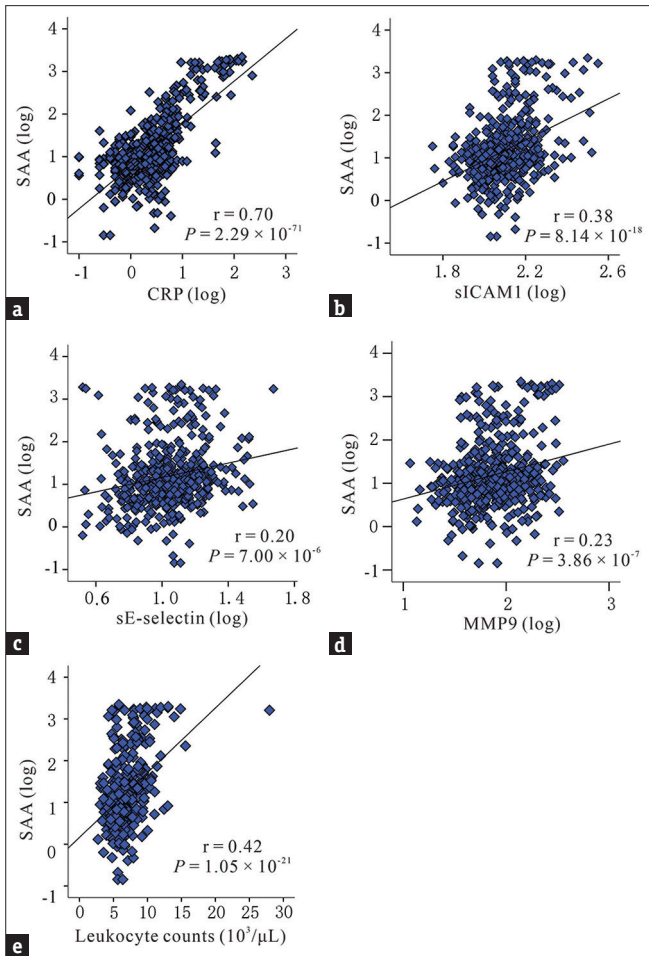
Figure 3: Association between SAA levels and circulating levels of inflammatory markers (a-d) and leukocyte counts (e) in patients with coronary artery disease. The levels were log-transformed before analysis
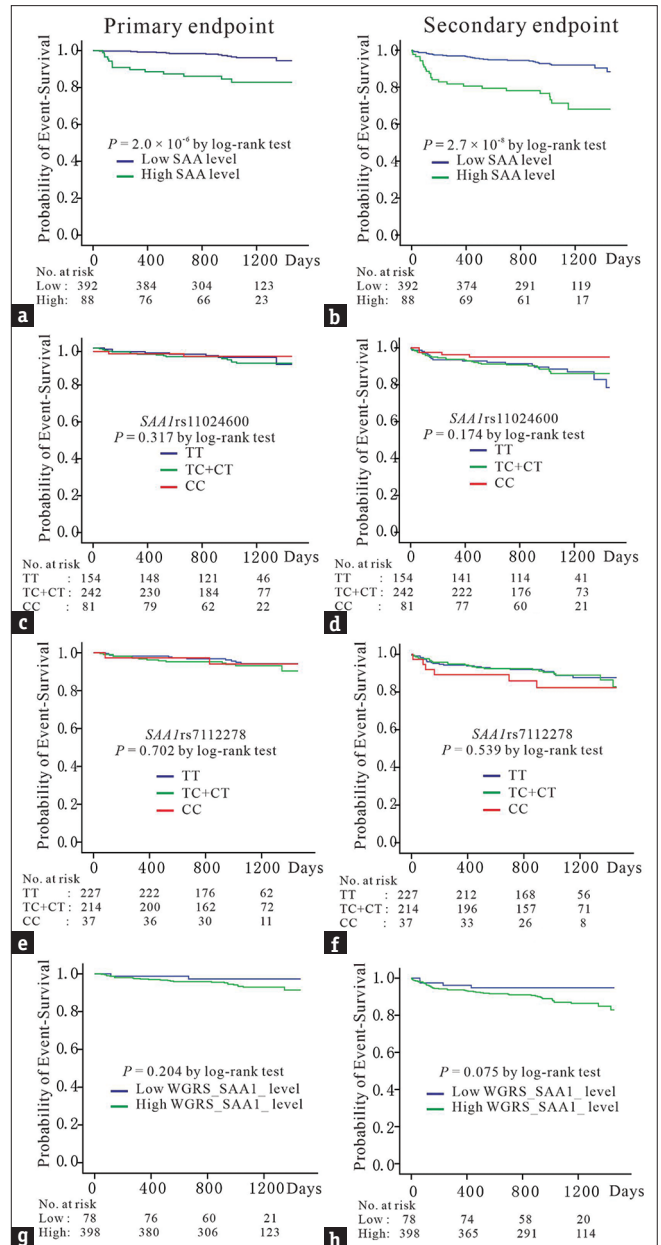

Figure 4: Kaplan–Meier curves of the cumulative incidence of primary and secondary endpoints in patients with coronary artery disease. Individuals are stratified according to SAA levels (>51.04 µg/mL vs. ≦51.04 µg/mL) (a and b), *SAA1* rs11024600 genotyping (c and d), *SAA1* 7112278 genotyping (e and f), and weighted genetic risk scores of *SAA1* genotypes (g and h) (>0.284 vs. ≦0.284) in patients with angiographically confirmed coronary artery disease. Significantly higher mortality and combined endpoints for coronary artery disease were noted for higher SAA levels. The study patients were followed for 1022 ± 320 days

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; $P = 2.0 \times 10^{-6}$ 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 4: Stepwise multivariate linear regression analysis of serum amyloid A levels in two study populations

	TWB participants			CAD patients		
	β	R^2	P	β	R^2	*P*
Age (years)	0.0050	0.0103	2.56×10^{-10}	-	-	-
Sex (female vs. male)	0.1124	0.0082	1.63×10^{-9}	-	-	-
BMI (kg/m²)	0.0271	0.0369	5.30×10^{-23}	−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^{-32}	−0.0176	0.0119	0.0001
Leukocyte counts (10³/μL)	0.0473	0.0140	2.84×10^{-16}	0.0631	0.0248	7.19×10^{-8}
HDL cholesterol level	0.5361	0.0139	4.85×10^{-9}			
ALT level	0.0018	0.0074	2.98×10^{-7}			
CRP (mg/L)				0.8333	0.4872	9.72×10^{-53}
sICAM1 (ng/mL)				0.8774	0.0132	1.26×10^{-5}
sE-selectin (ng/mL)				-	-	-
MMP9 (ng/mL)				-	-	-

TWB: Taiwan Biobank, CAD: Coronary artery disease, BMI: Body mass index, HDL: High-density lipoprotein, ALT: Alanine aminotransferase, sE-selectin: Soluble E-selectin, SAA: Serum amyloid A, sICAM1: Soluble intercellular adhesive molecule, MMP9: Matrix metalloprotease 9, CRP: C-reactive protein

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)			
HR (95% CI)	5.183 (2.436-11.029)	4.388 (1.989-9.684)	3.399 (1.517-7.616)
P	0.000019	0.00025	0.003
WGRS_*SAA1* (high vs. low)			
HR (95% CI)	2.1464 (0.584-10.403)	2.346 (0.547-10.066)	2.720 (0.617-11.991)
P	0.2198	0.2513	0.1861
Secondary endpoint			
SAA level (high vs. low)			
HR (95% CI)	4.083 (2.385-6.988)	3.472 (2.003-6.021)	2.721 (1.548-4.781)
P	2.885×10^{-7}	0.000009	0.001
WGRS_*SAA1* (high vs. low)			
HR (95% CI)	2.446 (0.883-6.777)	2.385 (0.854-6.660)	2.173 (0.772-6.116)
P	0.0855	0.0971	0.1418

Model 1: Unadjusted, Model 2: Adjusted for baseline data (sex, age, BMI, current smoking status, diabetes mellitus, hypertension, and with statin medication), Model 3: Adjusted for baseline data and initial presentation (sex, age, BMI, current smoking status, diabetes mellitus, hypertension, with statin medication) and four subgroups of initial presentation (stable angina pectoris, ACS/MI, CHF/lung edema, and others). CI: Confidence interval, HR: Hazard ratio, WGRS: Weighted genetic risk score, SAA: Serum amyloid A, ACS/MI: Acute coronary syndrome or myocardial infarction, CHF: Congestive heart failure, BMI: Body mass index

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.

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 patients with acute myocardial infarction. Ann Clin Biochem 1995;32 (Pt 2):196-200.

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: Implications for serum amyloid A function. Proc Natl Acad Sci U S A 1994;91:3186-90.

5. Rokita 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 antiinflammatory 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. A 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. Sorić Hosman I, Kos I, Lamot L. Serum amyloid A in inflammatory rheumatic diseases: A compendious 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, Hirschfield 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 AI, Rothenberg LS, DeBeer FC, Cantor RM, Rotter JI, Lusis 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, Hsu LA, Wu S, Teng 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, Juang JJ, Chiang FT, Teng MS, Lin JF, et al. Growth differentiation factor 15 May predict mortality of peripheral and coronary artery diseases and correlate with their risk factors. Mediators Inflamm 2017;2017:9398401.

25. Lin JF, Wu S, Juang JJ, Chiang FT, Hsu LA, Teng MS, et al. IL1RL1 single nucleotide polymorphism predicts sST2 level and mortality in coronary and peripheral artery 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. Er LK, Hsu LA, Juang JJ, Chiang FT, Teng MS, Tzeng IS, et al. Circulating chemerin 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 2019;25:6440-50.

32. Astle WJ, Elding H, Jiang T, Allen D, Ruklisa D, Mann AL, et al. The allelic landscape of human blood cell trait variation and links to common complex disease. Cell 2016;167:1415-29.e19.

33. Dehghan A, Dupuis J, Barbalic M, Bis JC, Eiriksdottir G, Lu C, et al. Meta-analysis of genome-wide association studies in>80 000 subjects identifies multiple loci for C-reactive protein levels. Circulation 2011;123:731-8.

34. Ridker PM, Pare G, Parker A, Zee RY, Danik JS, Buring JE, et al. Loci related to metabolic-syndrome pathways including LEPR, HNF1A, IL6R, and GCKR associate with plasma C-reactive protein: The women's genome health study. Am J Hum Genet 2008;82:1185-92.

35. Sabater-Lleal M, Huang J, Chasman D, Naitza S, Dehghan A, Johnson AD, et al. Multiethnic meta-analysis of genome-wide association studies in>100 000 subjects identifies 23 fibrinogen-associated Loci but no strong evidence of a causal association between circulating fibrinogen and cardiovascular disease. Circulation 2013;128:1310-24.

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. Circ 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, Yamen 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.
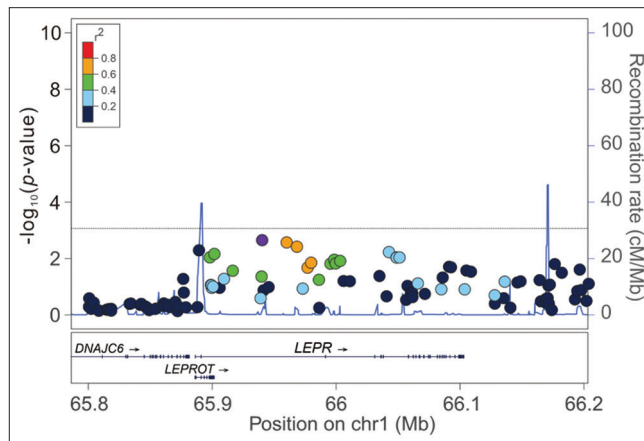
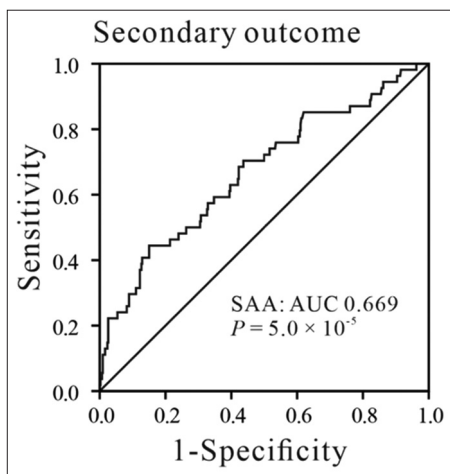# SUPPLEMENTARY METHOD

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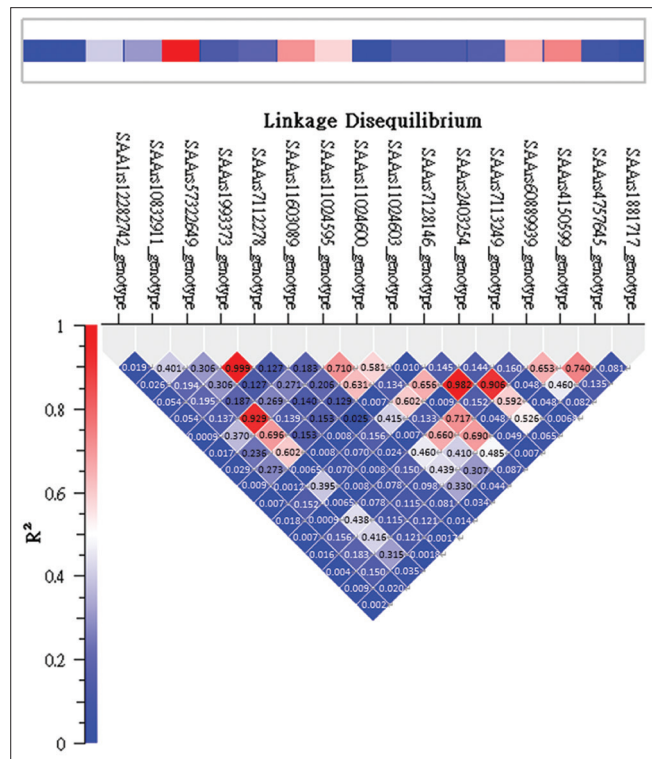
Supplementary Figure 1: Linkage disequilibrium of *SAA1* single-nucleotide polymorphisms

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 1: Inter- and intra-assay variability measures of the inflammatory markers

Biomarker	Patients with coronary artery disease**		
	Source	Intra-assay (%)	Inter-assay (%)
CRP (mg/L)	Plasma	CV=5.5	CV=6.5
SAA (µg/mL)*	Plasma	CV=6.1	CV=7.4
sE-selectin (g/L)*	Plasma	CV=5.8	CV=7.9
sICAM1 (g/L)*	Plasma	CV=4.6	CV=5.5
MMP9 (mg/L)*	Plasma	CV=2.3	CV=7.5

*In-house ELISA kits for SAA, sE-selectin, sICAM1, and MMP9 levels, **ELISA kits from R&D for SAA, sE-selectin, sICAM1, and MMP9 levels. ELISA: Enzyme-linked immunosorbent assay, sE-selectin: Soluble E-selectin, SAA: Serum amyloid A, sICAM1: Soluble intercellular adhesive molecule, MMP9: Matrix metalloprotease 9, CRP: C-reactive protein, CV: The coefficient of variation

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^{-5}	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^{-15}	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	$4.24×10^{-16}$
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**	G/A	0.305	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	$1.40×10^{-11}$
rs60889939	18352433	*GTF2H1*, Intron variant	G/A	0.425	−0.279	0.014	$1.11×10^{-84}$
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			TWB female		
	β	R^2	*P*	β	R^2	*P*
Age (years)	0.0033	0.0039	6.63E-03	0.0057	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