The minor C-allele of the rs2014355 variant in ACADS gene is associated with exercise-induced increase in HDL cholesterol levels in Taiwanese adults

Tzi-Peng Yang, PhD, Fen-Fen Shih, MS, Ming-Yi Hsu, PhD, Meng-Hsiun Tsai, PhD, Oswald Ndi Nfor, DVM, PhD, Pei-Hsing Chen, MS, Chien-Chang Ho, PhD, Chuan-Chao Lin, MD, PhD, Yung-Po Liaw, PhD

Abstract
We investigated the association between high-density lipoprotein cholesterol (HDL-C) and rs2014355 variant in the gene, short-chain acyl-coenzyme A dehydrogenase (ACADS) based on exercise habits.

Data collected between 2008 and 2015 for individuals aged 30 to 70 years were available in the Taiwan Biobank (TWB) database. Backward stepwise linear regression was used to evaluate the associations of rs2014355 and exercise with HDL-C levels.

We analyzed data of 5515 physically active and 4169 inactive biobank participants. The HDL-C concentrations were higher in the exercise compared to no exercise group (beta value, β = 1.79856; P < .0001). We observed that the test for interaction was significant for the ACADS rs2014355 variant and exercise (P for interaction = .0412). Multivariate analyses showed significant association between TC+CC genotype and HDL-C in the exercise (β = 1.09785; P value = .0146) compared to the no-exercise group (β = −0.03754, P = .9154).

In summary, the association between HDL-C and exercise differed significantly with respect to ACADS rs2014355 genotypes. Compared to the TT genotype, the TC+CC genotype together with exercise was associated with higher levels of HDL-C.

Abbreviations: ACADS = short-chain acyl-coenzyme A dehydrogenase, β = beta coefficient, BMI = body mass index, CI = confidence interval, HWE = Hardy-Weinberg equilibrium, MOST = Ministry of Science and Technology, SNP = single nucleotide polymorphisms, TWB = Taiwan Biobank.

Keywords: ACADS gene, exercise, HDL-C, SNP

1. Introduction
Short-chain acyl-coenzyme A dehydrogenase is a homotetrameric flavoenzyme that catalyzes the initial step of the mitochondrial fatty acid beta-oxidation pathway.[1,2] It cuts long-chain free fatty acids (FFA) into short-chains (C3/C4) when they are inside the mitochondria.[1,2,3] This pathway plays an important role in energy metabolism especially during a physiological response to tissue energy depletion during periods of fasting, illnesses, and increased muscular activity. Mutations of the ACADS gene are associated with deficiency of the short-chain acyl-coenzyme A dehydrogenase protein (SCADD) which is marked by an increased amount of fatty acids, and the presence of increased butyrylcarnitine (C4) in blood plasma, and increased ethylmalonic acid (EMA) concentrations in urine.[2,3,4]
It is well known that HDL-C has a strong correlation with the risk of coronary atherosclerosis.\textsuperscript{3,11,14} There is much evidence to show that exercise and diet are important in improving serum HDL-C levels, hence minimizing the risk of cardiovascular disease.\textsuperscript{1,5,10,12} It has also been reported that regular endurance exercise training may increase HDL-C levels.\textsuperscript{11,15} ACADS is one of the quantitative effectors of HDL-C.\textsuperscript{11} The mechanism underlying ACADS gene deletion in mice (Balb/cBy) showed higher levels of tance.\textsuperscript{2} Triglycerides, free fatty acids, cholesterol, glucose, and hepatic lipid were found to be lower in Balb/cBy mice.\textsuperscript{17} The ACADS gene is approximately 13 kb in length and has 10 exons at chromosome 12 (12q24.31).\textsuperscript{18} Several single nucleotide polymorphisms (SNPs) of this gene have been reported.\textsuperscript{1,18} In a genome-wide association study (GWAS), an association was found between ACADS rs2014355 SNP and the ratio between the short-chain acylcarnitines C3 and C4.\textsuperscript{9} Mirkov et al also found that ACADS SNPs affect serum metabolomics traits: mRNA of ACADS in liver tissue of people with rs2014355 TT genotype while 23.37% were those with the TT+CC genotype in the ACADS gene affects insulin release following oral glucose load.\textsuperscript{14} The above data imply that ACADS rs2014355 genotypes will affect glucose and lipid metabolism.

However, studies that explore the effects of exercise and ACADS on HDL-C are limited. The purpose of this study was to investigate the association of exercise and ACADS rs2014355 genotypes with HDL-C.

2. Material and methods

The entire data used in this study were retrieved from the TWB database (2008–2015). Enrollment in the Taiwan biobank is restricted to Taiwanese adults between the ages of 30 and 70 with no personal history of cancer. In our study, data were available for 9684 participants (that is, 5515 physically inactive and 4169 active participants). Baseline characteristics included exercise, rs2014355 (TT, TC+CC genotypes), sex (women/men), age, smoking habits (never/former/current), drinking habits (never/former/current), BMI (normal/underweight/overweight/obese), vegetarian (no/yes), coffee-drinking habits (no/yes), and disease (coronary heart disease, hyperlipidemia, diabetes, and hypertension) status (yes/no). We obtained exercise data through TWB questionnaires and defined exercise as participating in any exercise activity for over 30 minutes per session (at least 3 times per week) for the last 3 months. Other lifestyle variables used in our analysis have been previously described.\textsuperscript{10} Disease conditions including hyperlipidemia and coronary heart disease were determined using self-answered TWB questionnaires. Diabetes was determined by self-answered questionnaires and confirmed by a fasting blood glucose of over 126 mm Hg or HbA1c of over 6.5. Hypertension was also determined through questionnaires and confirmed by SBP and DBP of over 140 mm Hg and 90 mm Hg, respectively. All eligible TWB participants signed informed consent prior to data collection. The Institutional Review Board of Chung Shan Medical University Hospital approved this study (CS2–161114 and CS1–20009).

2.1. Statistical analysis

We performed statistical analyses using SAS version 9.4 (SAS Institute Inc., Cary, North Carolina, USA) and Plink 1.9. The rs2014355 SNP passed quality control (i.e., HWE P value ≥0.001, minor allele frequency = 0.12, and call rate = 0.999). HDL-C levels across different exercise groups were compared using the Student t-test. Differences between categorical variables were tested using the chi-square test. Backward stepwise linear regression was used to assess the associations of rs2014355 genotypes and exercise with HDL-C levels.

3. Results

Overall, 9,684 participants were enrolled in this study (Table 1). There were 5515 physically active (exercise group) and 4169 inactive individuals (no exercise group). HDL-C levels were significantly different between the exercise and no exercise group (P < 0.0001). The mean HDL-C concentration was 52.63 mg/dl for no exercise and 54.68 mg/dl for the exercise group. Of the participants in the no-exercise group, 76.63% were those with the rs2014355 TT genotype while 23.37% were those with the TC+CC genotype. Among those in the exercise group, 78% were women.

| Variables               | No exercise n = 5515 | Exercise n = 4169 | P value |
|-------------------------|----------------------|------------------|---------|
| HDL-C                   | 52.62±0.1799         | 54.67±0.2111     | <.0001  |
| rs2014355               |                      |                  |         |
| TT                      | 4226 (76.63)         | 3252 (78.00)     |         |
| TC+CC                   | 1289 (23.37)         | 917 (22.00)      |         |
| Sex                     |                      |                  | .1105   |
| Women                   | 2937 (53.25)         | 2152 (51.62)     |         |
| Men                     | 2578 (46.75)         | 2017 (48.38)     |         |
| Age                     |                      |                  | .0001   |
| Women                   | 46.87±0.1394         | 54.60±0.1579     | <.0001  |
| Men                     | 46.15±0.1367         | 54.60±0.1579     | <.0001  |
| Current                 | 749 (13.58)          | 292 (7.00)       |         |
| No                      | 4167 (75.56)         | 3260 (78.20)     |         |
| Former                  | 599 (10.86)          | 617 (14.80)      |         |
| Current                 | 461 (8.58)           | 295 (7.08)       |         |
| BMI                     |                      |                  | <.0001  |
| Normal                  | 2486 (45.08)         | 2072 (49.70)     |         |
| Underweight             | 180 (3.26)           | 74 (1.78)        |         |
| Overweight              | 1660 (30.10)         | 1278 (30.65)     |         |
| Obese                   | 1189 (21.56)         | 745 (17.87)      |         |
| Vegetarian              |                      |                  | .1686   |
| No                      | 5230 (94.83)         | 3979 (86.44)     |         |
| Yes                     | 285 (5.17)           | 190 (4.56)       |         |
| Coffee drinking habit   |                      |                  | <.0001  |
| No                      | 3529 (63.99)         | 2737 (65.65)     |         |
| Yes                     | 1986 (36.01)         | 1432 (34.35)     |         |
| Coronary heart disease  |                      |                  | .0024   |
| No                      | 5464 (99.08)         | 4102 (98.39)     |         |
| Yes                     | 51 (0.92)            | 67 (1.61)        |         |
| Hyperlipidemia          |                      |                  | <.0001  |
| No                      | 5209 (94.45)         | 3853 (92.42)     |         |
| Yes                     | 306 (5.55)           | 316 (7.58)       |         |
| Diabetes                |                      |                  | <.0001  |
| No                      | 4993 (90.53)         | 3642 (87.36)     |         |
| Yes                     | 522 (9.47)           | 527 (12.64)      |         |
| Hypertension            |                      |                  | <.0001  |
| No                      | 4529 (82.12)         | 3088 (74.07)     |         |
| Yes                     | 986 (17.88)          | 1081 (25.93)     |         |

Continuous variables are presented as mean±standard error and categorical variables as n (%).
those with the TT genotype while 22% were those with the TC+CC genotype. There was no significant difference in HDL-C concentrations of those with TT+CC and those with the TT genotype ($P=.1024$) (Table 2). However, HDL-C levels were significantly higher among individuals in the exercise, compared to no exercise group ($\beta=1.79856; P<.0001$). There was a significant interaction between ACADS rs2014355 and exercise on HDL-C concentration ($P=.0412$). Regression analysis showed a significant association between ACADS genotype and HDL-C only in the exercise group (i.e., $\beta=1.09785; P=.146$). The $\beta$-value was $-0.03754 (P=.9154)$ in the no-exercise group (Table 3). When no exercise and TT genotype was used as the reference group (Table 4), the $\beta$ value was $-0.02881, (P=.9372)$, for the TC+CC/no exercise group, 1.53846 ($P<.0001$) for the TT/exercise group, and 2.66237 ($P<.0001$) for the TC+CC/exercise group (Table 4).

### 4. Discussion

To our knowledge, this is the first study to examine the relationship between ACADS rs2014355 genotypes and HDL based on exercise habits. We found that the association between HDL-C and exercise differed with respect to the rs2014355 genotypes. Compared with the TT genotype, the TC+CC genotype together with exercise was more effective in improving HDL-C levels (that is, there was a 2.7 mg/dl increase in HDL-C levels of those with the TC+CC genotype compared to just 1.6 mg/dl among those with the TT genotype). In the absence of exercise, HDL-C concentrations were low and there were no differences regardless of the genotype.

A genome-wide GWAS carried out in Germany in 2010 pointed out that the metabolic capacity of the ACADS rs2014355C allele is stronger than that of the TT genotype. This is consistent with our study. Genes mRNA levels and their regulations/expressions are different in different tissues. Mirkov et al reported that the ACADS mRNA levels in liver tissues were higher among TC+CC (rs2014355) than in TT individuals. ACADS is highly expressed in the small intestine, colon, and liver, but there is no data to show its expression in white blood cells. In Taiwan biobank, DNA was extracted from white blood cells.

So far, it is well known that exercise can improve the performance of ACADS, HDL-C, and insulin sensitivity. Our results suggest that this phenomenon could be more pronounced in those with the minor C allele. Besides, studies by Hornbak et al in 2011 showed that the minor C-allele of rs2014355 was associated with reduced glucose-stimulated insulin released during an oral glucose tolerance test (OGTT) and may be mediated through an impaired $\beta$-oxidation of fatty acids. This study also indicated that levels of HDL-C under OGTT did not differ between people with TT or TC+CC genotypes. In contrast, the studies cited above did not include exercise in their study design, as we have done. Of note, exercise plays a major role in the regulation of ACADS.

Our study was limited in that information on the intensity and frequency of exercise was not available in the Taiwan Biobank.
### Table 4

**Association of HDL-C based on exercise habits and rs2014355 genotypes.**

| Variables                                | β     | P value |
|------------------------------------------|-------|---------|
| Exercise, rs2014355 (ref: No exercise, TT) | -0.02881 | .9372   |
| No exercise, TC+CC                        | 1.53846 | <.0001  |
| Exercise, TT                             | 2.66237 | <.0001  |
| Sex (ref: Women)                          |       |         |
| Men                                       | -7.68390 | <.0001  |
| Age                                       | 0.04146 | .0007   |
| Cigarette smoking habit (ref: No)         |       |         |
| Former                                    | -0.74611 | .0570   |
| Current                                   | -3.29365 | <.0001  |
| Alcohol drinking habit (ref: No)          |       |         |
| Former                                    | -0.75567 | .2600   |
| Current                                   | 3.35266 | <.0001  |
| BMI (ref: Normal)                         |       |         |
| Underweight                               | 7.42941 | <.0001  |
| Overweight                                | -5.15605 | <.0001  |
| Obese                                     | -8.12641 | <.0001  |
| Vegetarian (ref: No)                      |       |         |
| Yes                                       | -6.19380 | <.0001  |
| Coffee drinking habit (ref: No)           |       |         |
| Yes                                       | 1.05097 | <.0001  |
| Coronary heart disease (ref: No)          |       |         |
| Yes                                       | -2.21828 | .0404   |
| Hyperlipidemia (ref: No)                  |       |         |
| Yes                                       | -1.70297 | .0006   |
| Diabetes (ref: No)                        |       |         |
| Yes                                       | -3.92081 | <.0001  |
| Hypertension (ref: No)                    |       |         |
| Yes                                       | -0.93990 | .025    |

dataset. Further investigations in this area are recommended. HDL-C levels might be influenced by exercise type and intensity. This study adds more knowledge on exercise-related HDL-C elevation to improve cardiovascular disease.

### 5. Conclusions

The association between HDL-C and exercise differed based on the ACADS rs2014355 genotypes. Compared to the TT genotype, the TC+CC genotype in combination with exercise was associated with higher levels of HDL-C. These results suggest that people with the rs2014355 TT genotype may improve their HDL-C levels by increasing their physical activity. This might in turn help to reduce the risk of cardiovascular disease.

### Author contributions

**Conceptualization:** Tzi-Peng Yang, Fen-Fen Shih, Ming-Yi Hsu, Meng-Hsiun Tsai, Oswald Ndi Nfor, Pei-Hsing Chen, Chien-Chang Ho, Chuan-Chao Lin, Yung-Po Liaw.

**Data curation:** Oswald Ndi Nfor, Pei-Hsing Chen, Chien-Chang Ho.

**Formal analysis:** Pei-Hsing Chen.

**Methodology:** Tzi-Peng Yang, Fen-Fen Shih, Ming-Yi Hsu, Meng-Hsiun Tsai, Oswald Ndi Nfor, Pei-Hsing Chen, Chien-Chang Ho, Chuan-Chao Lin, Yung-Po Liaw.

**Supervision:** Chuan-Chao Lin, Yung-Po Liaw.

**Validation:** Tzi-Peng Yang, Fen-Fen Shih, Ming-Yi Hsu, Meng-Hsiun Tsai, Yung-Po Liaw.

**Writing – original draft:** Tzi-Peng Yang.

**Writing – review & editing:** Fen-Fen Shih, Ming-Yi Hsu, Meng-Hsiun Tsai, Oswald Ndi Nfor, Pei-Hsing Chen, Chien-Chang Ho, Chuan-Chao Lin, Yung-Po Liaw.

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