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Interaction between peroxisome proliferator activated receptor δ and epithelial membrane protein 2 polymorphisms influences HDL-cholesterol levels in the Chinese population

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Summary

PPARs are transcription factors involved in the regulation of key metabolic pathways. Numerous *in-vivo* and *in-vitro* studies have established their important roles in lipid metabolism. A few SNPs in *PPAR* genes have been reported to be associated with lipid levels. In this study, we aimed to

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DISCLOSURES

The authors declare no conflict of interest.

investigate the interactive effects between SNPs in three *PPAR* isoforms α/δ/γ and other genetic variants across the genome on plasma high-density lipoprotein-cholesterol (HDL-C) levels. Study subjects (N = 2,003) were genotyped using Illumina HumanOmniZhongHua-8 Beadchip. Fifty-three tag SNPs +/− 100kb of *PPAR* α, δ, and γ ($r^2 < 0.2$) were selected. The effect of interactions between *PPAR* SNPs and those across the genome on HDL-C were tested using linear regression models. One statistically significant interaction influencing HDL-C was detected between *PPARδ* SNP rs2267668 and epithelial membrane protein 2 (*EMP2*) downstream SNP rs7191411 (N = 1,993, β = 0.74, adjusted P = 0.022). This interaction was successfully replicated in the meta-analysis of two additional Chinese cohorts (N = 3,948, P = 0.01). The present study showed a novel SNP×SNP interaction between rs2267668 in *PPARδ* and rs7191411 in *EMP2* that has significant impact on circulating HDL-C levels in the Singaporean Chinese population.

Keywords

HDL-C; PPAR; interaction; SNP; Genetics; Lipoproteins

INTRODUCTION

Cardiovascular disease is the major cause of mortality and morbidity worldwide. HDL-C has been overwhelmingly demonstrated as a key factor that is inversely and independently associated with the risk of cardiovascular disease in epidemilogical studies (Emerging Risk Factors *et al.*, 2009, Durrington, 2002). The protective effect of HDL-C has been demonstrated to be mediated through the reverse cholesterol transport (Kwiterovich, 1998, Barter *et al.*, 2004), which results in the movement of cholesterol from various tissues to the liver. PPARs are well-known lipid-activated transcription factors that play a crucial role in the regulation of key molecules in reverse cholesterol transport. PPAR has three isoforms, α, δ and γ, which are structually related but differ in expression profiles and target genes. PPARα is abundantly expressed in the liver and enterocytes. Its activation increases HDL-C levels (Millar, 2013, Shah *et al.*, 2010). PPARγ is predominantly expressed in adipose tissues and plays an important role in cholesterol homeostasis (Evans *et al.*, 2004). It controls the expression of ATP-binding cassette A1 and caveolin 1, thus contributing to variations in HDL-C levels (Akiyama *et al.*, 2002, Zhao *et al.*, 2008, Llaverias *et al.*, 2004). Unlike PPARα and PPARγ, PPARδ is ubiquitously expressed but only at low levels in the liver (Higgins *et al.*, 2012). Studies of PPARδ have revealed that it promotes reverse cholesterol transport through its action on increasing the number of HDL-C particles (Oliver *et al.*, 2001). Given the substantial role of PPARs in lipid metabolism, various agonists of PPARs have been synthesized and the biological effects of PPAR agonists on lipids and cardiovascular diseases have been extensively tested *in vivo* and *in vitro*. For example, the PPARδ agonist MBX-8025 and GW501516 have been studied in randomized trials in humans, showing an effect of increased HDL-C and reduced low density lipoprotein-cholesterol (LDL-C) (Choi *et al.*, 2012, Olson *et al.*, 2012). Hence, PPARs are important factors that can increase HDL-C levels and reduce the risk of cardiovascular disease (Jay & Ren, 2007, Duval *et al.*, 2002, Flavell *et al.*, 2000). However, a phenomenom called “disappearing HDL syndrome”, which is a reversible severe HDL-C deficiency, has also been observed in some subjects after PPAR agonist treatment (Goldberg & Mendez, 2007,

Keidar *et al.*, 2007, Sarker *et al.*, 2004, Senba *et al.*, 2006). This perhaps indicates specific inherent inter-individual variability to PPAR treatment response, such as epistatic interactions that have yet to be understood. Moreover, it has been estimated that 40 to 60% of plasma lipid variance is genetically determined (Namboodiri *et al.*, 1985, Weissglas-Volkov & Pajukanta, 2010). However, the 157 recently identified loci could only explain less than 13% of variation for HDL-C (Global Lipids Genetics *et al.*, 2013, Asselbergs *et al.*, 2012). It is therefore believed that part of the missing heritability could be attributed to gene-environment and gene-gene interactions. Nevertheless, the heavy computational and statistical burden present great challenges in identifying novel interactions. Effective filtering strategies have been suggested to reduce the number of data. One approach is using statistically significant SNP to prioritize promising SNPs (Evans et al., 2006, Kooperberg & Leblanc, 2008). Another approach is using intrinsic or extrinsic knowledge to reduce the number of testing (Xenarios et al., 2000, Stark et al., 2006, Greene et al., 2009, Moore & White, 2007, Kanehisa & Goto, 2000, Bush et al., 2009). In this study, we selected PPARs based on prior extrinsic knowledge. As there have been no prior studies exploring how genetic variants in the three *PPAR* isoforms (α/δ and γ) interact with other SNPs across the genome to impact on HDL-C levels, we aimed to fill this knowledge gap using Asian datasets from Singapore.

MATERIALS AND METHODS

Singapore Chinese Health Study

The Singapore Chinese Health Study (SCHS) was used as the discovery cohort. It is a population-based prospective cohort which began in 1993 and has a recruitment of 63,257 residential Singaporean Chinese between age 45–74 by 1998 (Hankin *et al.*, 2001). The cohort study recruited only participants belonging to one of the two major Chinese dialect groups in Singapore, the Hokkiens or the Cantonese, who originated from two contiguous prefectures in southern China. Subjects were interviewed face-to-face at their home by trained interviewers using a well-structured questionnaire, which sought information on basic demographics, smoking status, usual physical activity, food consumption, menopausal status (women only), medical history, and family history (Hankin *et al.*, 2001). Cases that had fatal coronary heart disease (CHD) or suffered from non-fatal myocardial infarction (MI) were identified through the Singapore Registry of Births and Deaths and the Hospital Discharge Database or the Singapore Myocardial Infarction Registry (SMIR) respectively. For all non-fatal cases identified through the Hospital Discharge Database, medical records were retrieved and reviewed by a cardiologist and only those who had confirmed MI using the Multi-Ethnic Study of Atherosclerosis criteria (available at: http://www.mesa-nhlbi.org/manuals.aspx), were included. The SMIR uses similar methods to verify cases through medical record review (Koh *et al.*, 2011). Each verified MI or CHD case were matched with two SCHS pariticipants who were alive and free of CHD at the time of the MI diagnosis or CHD death on sex, dialect group, year of birth (± 2 years), year of recruitment (± 1 year) and date of blood collection (± 6 months). All participants were given written informed consents. This study were approved by National Health Group Domain Specific Review Board and National University of Singapore Institutional Review Board.

Significant findings in SCHS were then evaluated in four other cohorts. They are the Singapore Chinese Eye Study (SCES)(Lavanya *et al.*, 2009), the Singapore Malay Eye Study (SiMES) (Foong *et al.*, 2007), the Singapore Indian Eye Study (SINDI) (Lavanya *et al.*, 2009) and the Singapore Prospective Study Program (SP2) (Nang *et al.*, 2009).

Singapore Epidemiology of Eye Disease (SEED) studies

The SCES, SiMES and SINDI are population-based, cross-sectional studies of Singaporean Chinese, Malay and Indian aged 40 to 80 years (Lavanya *et al.*, 2009, Foong *et al.*, 2007, Sabanayagam *et al.*, 2015, Cheung *et al.*, 2014). They were all conducted by the Singapore Eye Research Institute and commenced between 2004 and 2007. All subjects were selected using age-stratified (10 year age group) random sampling strategy from a computer-generated list provided by the Ministry of Home Affairs. Selected subjects underwent an extensive examination procedure and interviews with detailed questionnaires (Lavanya *et al.*, 2009, Foong *et al.*, 2007, Sabanayagam *et al.*, 2015, Cheung *et al.*, 2014). Non-fasting blood samples were collected for laboratory analysis including serum lipids. All participants gave their written informed consents. The studies followed the principles of the Declaration of Helsinki and approved by the Singapore Eye Research Institute Institutional Review Board. The detailed methodology of the three studies have been previously published (Lavanya *et al.*, 2009, Foong *et al.*, 2007, Sabanayagam *et al.*, 2015, Cheung *et al.*, 2014).

Singapore Prospective Study Program

The SP2 is a repeat examination of 7,742 subjects (74.1% response rate) drawn from 4 population-based, cross-sectional surveys conducted in Singapore-Thyroid and Heart Study, the National Health Survey 1992, the National University of Singapore Heart Study and the National Health Survey 1998, which have been described before (Nang *et al.*, 2009). Data on demographic, life style factors and medical history were collected by interviewer-administered questionnaires. The likely MI/CHD status was determined based on their responses (No, Yes) to the following questions in the questionnaire, “Has your doctor ever told you that you have blockage of the arteries to your heart” or “Have you had ever had a heart attack”. Subjects were aged 18–69 at baseline and represented a random sample of the Singapore population. Among participants, 5,094 provided blood samples and overnight fasting blood samples. Informed consents were obtained from all participants. This study was approved by National University of Singapore Institutional Review Board and Singapore General Hospital Institutional Review Board.

Blood collection and lipoprotein measurements

In SCHS, blood samples were collected during home visits. The final number of blood samples available for the SCHS was 28,439; including MI cases for whome blood was taken prior to their incident event (Hankin *et al.*, 2001). In SCES, SiMES, SINDI and SP2, blood samples were collected at the time participants visited study clinic for examination (Lavanya *et al.*, 2009, Nang *et al.*, 2009). Blood components of each sample (i.e., fractions of plasma, buffy coat, serum and red blood cells) were separated and stored at −80 °C.

Non-fasting total cholesterol, HDL-C, LDL-C and triglycerides were measured in SCHS, SCES, SiMES and SINDI samples at baseline with the enzymatic, colorimetric method or

elimination/catalase method using the Siemens Advia 2400 instrument (Siemens Medical Solutions Diagnostics, Deerfield, IL, USA) (Nang *et al.*, 2009, Foong *et al.*, 2007, Lavanya *et al.*, 2009). Fasting total cholesterol, HDL-C and triglycerides were measured in SP2 samples with kits from Boehringer Mannheim Systems (Mannheim, Germany) and a BM/Hitachi 747 analyzer (Roche Diagnostics, Corp. Indianapolis, IN) (Nang *et al.*, 2009, Foong *et al.*, 2007, Lavanya *et al.*, 2009). LDL-C was calculated using the Friedewald formula in SP2. Lipid-lowering medication was not available for the SCHS. In total, 2,003 subjects in SCHS, 2,099 subjects in SP2, 1,872 subjects in SCES, 2,541 subjects in SiMES and 2,538 subjects in SINDI were available for subsequent association analyses.

All the measurements were undertaken at National University Hospital Referral Laboratories, which participated in external quality assessment schemes such as the National Proficiency Testing Programmes, CAP, Bio-Rad Laboratories EQAS, QASI, RCPA and UKNEQAS. No significant deviation in measurements of reference samples was observed.

Genotyping and quality control

SCHS, SCES, SiMES, SINDI and SP2 were genotyped on different arrays, with SCHS on Illumina HumanOmniZhongHua-8 BeadChip (San Diego, California, the United States), 1/3 SCES, SiMES, SINDI and 1,467 samples of SP2 on the Human610-Quad BeadChip, 2/3 SCES on Illumina OminiExpress, and 1,016 samples of SP2 on the Human 1M-Duo v3 BeadChip. The quality control of SCES, SiMES SINDI and SP2 have been described elsewhere (Dorajoo *et al.*, 2013, Liao *et al.*, 2014). Chip-wise quality control procedures have been conducted following standard criteria in all studies (Supplementary Table I and II). Briefly, SNP quality control was conducted based on allele frequency (MAF < 0.01), call-rates (< 0.95) and deviations from Hardy-Weinberg Equlibrium ($P < 10^{-04}$). Sample quality control was conducted based on sample call rates (< 0.98), heterozygosity (> 3S.D), first degree relateness and discordant ethnic relationship based on Principle component analyses. After quality control, 2003 samples and 802,635 SNPs remained in SCHS. Linkage disequilibrium (LD) based pruning ($r^2 < 0.2$) was applied on the genome-wide autosomal SNPs using PLINK (version 1.07) in SCHS. Finally, 142,208 independent SNPs remained in SCHS for further analysis.

Candidate SNP selection

The genomic locations of the three *PPAR* genes were obtained from Ensembl Genome Brower (GRCh37/hg19, http://www.ensembl.org/index.html). The SNPs 100kb upstream and downstream of these genes were extracted by Haploview 4.2 using CHB+JPT analysis panel (Version 3 Release R2). The number of SNPs for *PPAR* α, δ, γ were 227, 110 and 163, respectively. Of this total number of 500 *PPAR* SNPs, 233 SNPs were part of the 802,635 SNPs that were genotyped on Illumina ZhongHua array which passed the quality control. These genotyped SNPs were further pruned by selecting those with $r^2<0.2$ using PLINK version 1.07. Finally, 53 independent SNPs of *PPAR* (25 in *PPARα*, 10 in *PPARδ* and 18 in *PPARγ*) remained for analysis (Supplymentary Table III).

MicroRNA binding site prediction

PolymiRTS Database 3.0 (http://compbio.uthsc.edu/miRSNP/) was utilized to predict the effects of SNPs on miRNA taget sites in our study (Bhattacharya *et al.*, 2014).

LD pattern comparison

The online database Singapore Genome Variation Project (SGVP, http://www.statgen.nus.edu.sg/cgi-bin/gbrowse/sgvp/#search) was utilized to compare the LD pattern around SNPs among different ethnic groups (Teo *et al.*, 2009).

Statistical analysis

Statistical analysis of data was carried out with STATA (version 12.0). Differences between means in demographic and biochemical parameters were evaluated by the *t*-test. Differences in frequencies between groups were examined by the chi-square test. Raw HDL-C levels were normalized by rank-based inverse normal transformation in all cohorts (Beasley *et al.*, 2009). Samples with more and less than 3 S.D of rank-based inverse normal transformated HDL-C levels were excluded from analyses. The number of outliers were 6 in SCHS, 5 in SCES, 5 in SP2, 6 in SiMES and 6 in SINDI. Body mass index (BMI) was derived from height and weight measurements (BMI = weight/height^2). Missing BMI values were imputed using multiple imputation in the SCHS dataset only (White *et al.*, 2011). The association of genetic variants with HDL-C was evaluated in SCHS using PLINK version 1.07 (Purcell *et al.*, 2007) using a linear regression model with adjustment of age, age^2, gender, smoking status, MI status and imputed BMI. To adjust the multiple tests in 53 SNPs, we defined the significance for association as $< 9.43\times10^{-4}$. Interaction was first tested between 53 PPAR SNPs and 142,208 independent genome wide SNPs in an additive model in SCHS (N = 2,003). This was executed in PLINK version 1.07 using a linear regression model by including the multicative term of 2 SNPs (each of the 53 *PPAR* SNPS*each of the 142,208 genomewide SNPs). Three rare double homozygotes of *PPARδ* and *EMP2* were observed among the five cohorts (N = 10,973). To reduce the possibility of a chance finding caused by rare double homozygotes, the interactions were presented in a dominant model with adjustment for age, age^2, gender, BMI, smoking status, priciple components (for non-Chinese cohorts) lipid-lowering medication and MI status where available. We defined the statistical significance as 6.63×10^{-9} based on 7,537,024 tests (53 *PPAR* SNPS*142,208 genomewide SNPs). Replication analyses of top interaction hits identified from the SCHS were conducted in SCES, SP2, SiMES and SINDI using the same model. Meta-analysis was conducted in the two Chinese cohorts, SCES and SP2, using fixed effect model. One way analysis of variance and Tukey's honestly significant difference pos-hoc test were used for the multiple comparisons of genotypic mean HDL-C levels between different combined genotypes of rs2267668 (*PPARδ*) and rs7191411 (*EMP2*).

RESULTS

As shown in Table 1, gender, age, HDL-C and BMI levels varied significantly across the five Singaporean cohorts. We therefore adjusted for age, gender and BMI in subsequent HDL-C association analyses. We also adjusted for smoking status, as it is a strong determinant of HDL-C levels.

The associations of 53 *PPAR* tag SNPs with HDL-C in SCHS were examined by linear regression analysis. None of them remained significant after correction for multiple testing at a threshold *P* value of 9.43×10^{-4} (Supplementary Table IV). Additionally, these 53 SNPs did not show significant main effect on other lipid traits including total cholesterol, LDL-C and triglycerides (data not shown). The gene-gene interactions were subsequently tested between *PPAR* SNPs and other independent SNPs across genome using an additive model. We observed one significant gene-gene interaction between *PPARδ* (rs2267668) and *EMP2* (rs7191411) (β = 0.58, unadjusted $P = 1.12 \times 10^{-10}$, adjusted $P = 8.44 \times 10^{-04}$, Table 2) in the additive model. To reduce the possibility of a chance finding caused by one double rare homozygote (HDL-C level = 2.49mmol/L, $N = 1$) and the bias caused by MI status, the interaction was examined in a dominant model with stratification of MI status and remained significant (β = 0.74, unadjusted $P = 2.97 \times 10^{-09}$, adjusted $P = 0.022$) (Table 2). This interaction was subsequently examined in four additional Singaporean datasets, SCES, SP2, SiMES and SINDI, and was successfully replicated in the meta-analysis of the two Chinese cohorts (Table 2; $P = 0.01$) SCES and SP2, but not in the non-Chinese cohorts SiMES (β = −0.11, $P = 0.29$) and SINDI (β = −0.14, $P = 0.15$) (Table 2).

Figure 1 shows the HDL-C-lowering effect of increasing number of minor alleles from either one of the *PPARδ* and *EMP2* SNP. However, plasma HDL-C levels were significantly elevated among subjects when minor alleles from both SNPs are present. The levels of HDL-C for each genotype combination are presented in Table 3 for the discovery and two replication Chinese cohorts.

The *EMP2* variant rs7191411 is 339 bases downstream of *EMP2*. To explore potentially functional SNPs that are in linkage disequilibrium (LD) with this SNP ($r^2 > 0.90$), we calculated pairwise LD of all genotyped SNPs across the chromosome 16 with rs7191411. One such SNP, rs12928798 ($r^2 = 0.97$ in SCHS, $r^2 = 1$ for all Chinese subgroups in 1000 Genome database), was identified. SNP rs12928798 is located in the 3'UTR region of *EMP2*. The *PPARδ* SNP rs2267668 is located within the intronic region of *PPARδ*. No potentially functional SNPs in LD with this SNP could be found in the genotyped SNPs.

DISSCUSSION

In this study, we first examined the association of 53 common variants in the three *PPAR* genes with HDL-C for their main effects. Subsequently, the interactions between the *PPAR* variants and all independent genotyped SNPs elsewhere in the genome were analyzed. None of the 53 SNPs were significantly associated with HDL-C levels after adjustment for multiple comparisons. However, one statistically significant interaction between *PPARδ* (rs2267668) and *EMP2* (rs7191411) was identified, which showed an increase of HDL-C levels in individuals carrying minor alleles from both SNPs.

The *EMP2* SNP tested in this study (rs7191411) is in high LD with rs12928798, which has been predicted to be a binding site of hsa-miR-4302 by PolymiRTS Database 3.0. Therefore, the observed interaction effect of rs7191411 may be attributed to its high LD with the latter, which could influence the expression level of *EMP2*. Subsequent *in vivo* and *in vitro* studies

would be necessary to elucidate the mechanism of how *PPARδ* and *EMP2* interact to affect HDL-C levels.

The *PPARδ* and *EMP2* interaction was replicated among two independent Singaporean Chinese cohorts but not among the Malay and Indian datasets. The effect of the interactions in all three Chinese cohorts were consistent (all showing positive betas). One plausible explanation for the failure of replication in the Malays and Indians could be due to differing LD patterns at the two identified loci (*PPARδ* and *EMP2*) among various ethnic groups evaluated in the study. Based on data from haplotype maps of Chinese, Malay, Indian and European population groups that was made available by the Singapore Genome Variation Project (http://www.statgen.nus.edu.sg/cgi-bin/gbrowse/sgvp/#search) (Teo *et al.*, 2009), there is evidence of a different pattern of LD around rs2267668 (*PPARδ*) between Chinese and Indian population groups but not between Chinese and Malays, and between Chinese and Europeans (Supplementary Figure I). We are not able to account for the failure of replication in the Malays. This is one of the limitation of our study. The other limitation is the lack of data for lipid-lowering medication in SCHS and verified MI status in SP2 and SEED studies.

PPARs are well-known lipid-activated transcription factors. Many studies have shown the effects of interactions between PPAR polymorphisms and alcohol consumption, dietary polyunsaturated fatty acid, diet and physical activity on serum lipid levels (Wei *et al.*, 2011, Chan *et al.*, 2006, Robitaille *et al.*, 2007, Halder *et al.*, 2014). However, only a few studies have investigated the interactions between PPAR polymorphisms and other genetic variants on serum lipid levels. These studies focused on the gene-gene interactions within PPAR receptors, showing a gender or diet conditioned interaction between PPAR δ polymorphism rs2016520 and the PPAR α polymorphism rs1800206 on LDL-C levels (Skogsberg *et al.*, 2003, Alsaleh *et al.*, 2011).

PPARδ is a ubiquitously expressed transcription factor. Cellular and animal studies have shown that an agonist of PPARδ (GW501516) could lead to a 80% increase of HDL-C compared to baseline level through ATP binding cassette transporter subfamily A member 1 (Oliver *et al.*, 2001, Barish *et al.*, 2008). Recently, several clinical trials have demonstrated that patients dosed with PPARδ agonist showed an enhancement of HDL-C in terms of levels and the number of HDL particles (Ooi *et al.*, 2011, Olson *et al.*, 2012, Choi *et al.*, 2012). The mechanism underlying the effect of PPARδ on HDL-C is through the protection of caveolin-1, an essential protein in reverse cholesterol transport, from degradation (Her *et al.*, 2013). This protein has been reported to localize and interact with ATP binding cassette transporter subfamily A member 1 and their interaction is crucial for caveolin-1 regulation of cholesterol efflux (Lin *et al.*, 2009). Interestingly, another member of the PPAR family, PPARγ, has been reported to modulate the expression level of caveolin-1(Burgermeister *et al.*, 2003). This suggests that PPARδ and PPARγ play important roles in cholesterol efflux through the regulation of caveolin-1 in protein and transcriptional level. Importantly, EMP2 has been demonstrated to down regulate caveolin-1(Forbes *et al.*, 2007, Wadehra *et al.*, 2004). A recent report has shown that the mutation in EMP2 can cause childhood-onset nephrotic syndrome, of which hyperlipidemia is one of the cardinal manifestations (Gee *et al.*, 2014). Although the biological relationship between EMP2 and PPARs has never been

studied, our study provides statistical evidence suggesting that EMP2 and PPARδ could interact to influence cholesterol efflux and thus modulate HDL-C. The relevant biological roles of PPARδ and EMP2 in reverse cholesterol transport also provided some support to the possibility of such an interaction. The overall impact of this interaction may not be limited to the regulation of HDL-C concentrations but could also fundamentally affect the cholesterol efflux capacity, which has been shown to be a more important factor in the prediction of coronary artery disease than HDL-C concentrations per se (Khera *et al.*, 2011).

PPARδ agonists, including Vascepa (Caldari-Torres *et al.*, 2006, Kondo *et al.*, 2007), Bezafibrate (Tenenbaum *et al.*, 2005), Treprostinil (Ali *et al.*, 2006), MBX-8025 (Choi *et al.*, 2012) and GW501516 (Olson *et al.*, 2012), are effective and promising drugs for lipid disorders and have shown properties of raising HDL-C and lowering LDL-C. The three drugs Vascepa, Bezafibrate, Treprostinil are FDA-approved commercial drugs. MBX-8025 and GW501516 are currently under phase II clinical evaluations (NCT00158899, NCT00841217, NCT00388180, NCT00701883, and NCT02472535). However, the clinical effects of PPAR agonist treatment may be complicated, an example being the “disappearing HDL syndrome” (Goldberg & Mendez, 2007, Keidar et al., 2007, Sarker et al., 2004, Senba et al., 2006). As PPARs function as transcription factors, any molecular interactions with PPARs might influence the final clinical effect of PPAR agonists. The identification of EMP2 in this study as a potential molecule that may interact with PPARδ may provide a lead for future investigations to unravel the puzzling effects of PPAR agonists in clinical use.

In conclusion, our study provides genetic epidemiological evidences for the first time that *PPARδ* (rs2267668) could interact with *EMP2* (rs7191411) to influence circulating HDL-C levels. A significant reduction of HDL-C levels was observed in subjects with minor allele(s) from either SNPs but an increase of HDL-C when minor alleles from both SNPs are present.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviation

CHD	coronary heart disease
EMP2	epithelial membrane protein 2
LD	linkage disequilibrium
MAF	minor allele frequency
MI	myocardial infarction
SCES	Singapore Chinese Eye Study
SCHS	Singapore Chinese Health Study
SEED	Singapore Epidemiology of Eye Disease
SGVP	Singapore Genome Variation Project
SiMES	Singapore Malay Eye Study
SINDI	Singapore Indian Eye Study
SMIR	Singapore Myocardial Infarction Registry
SP2	Singapore Prospective Study Program
UTR	untranslated region

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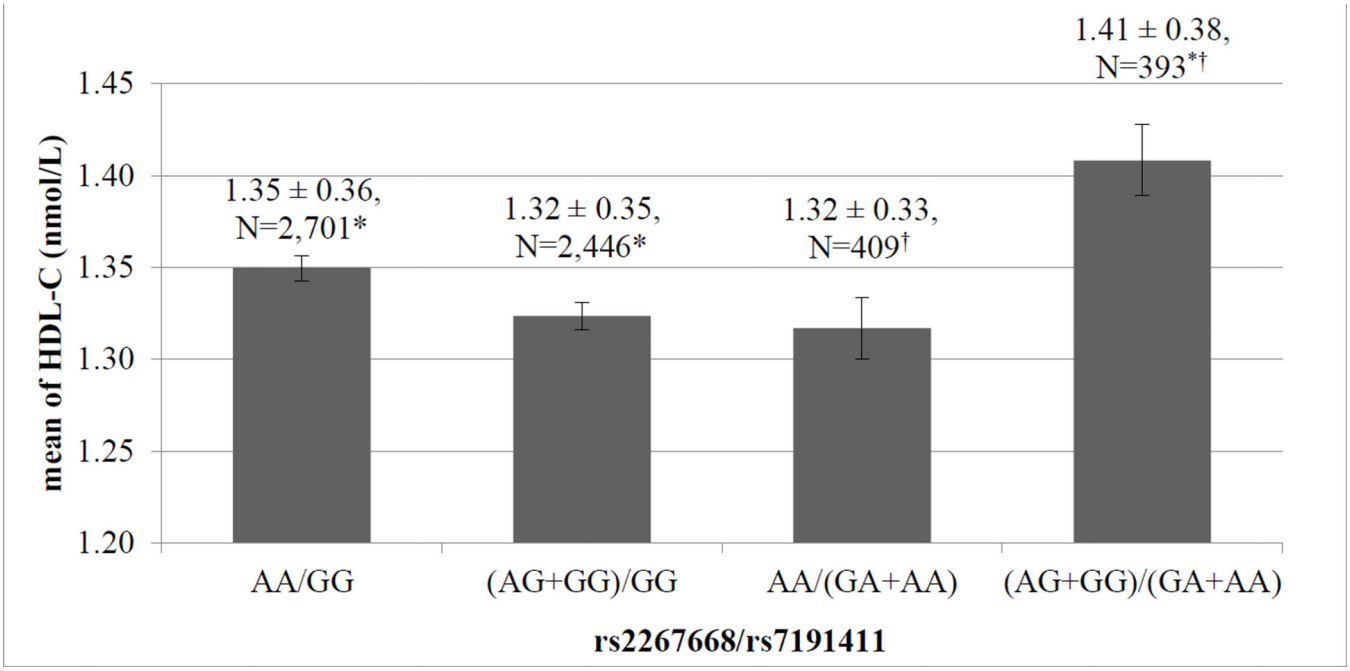

Figure 1.

Table 1

Demographic characterstics of the five Singaporean cohorts

Studies	SCHS (N=2,003)	SP2 (N=2,099)	SCES (N=1,872)	SiMES (N=2,541)	SINDI (N=2,538)
Ethnicity	Chinese	Chinese	Chinese	Malay	Indian
Men (%)	63.95	41.78	51.34	49.43	51.14
Smoker (%)[a]	42.49	16.72	26.94	39.64	26.96
Age (years)	66.42 ± 7.82	47.72 ± 10.97	58.46 ± 9.54	59.08 ± 11.04	58.04 ± 10.01
BMI (kg/m^2)[b]	22.98 ± 3.35	22.74 ± 3.70	23.76 ± 3.54	26.38 ± 5.11	26.12 ± 4.98
HDL-C (mmol/L)	1.32 ± 0.32	1.40 ± 0.36	1.30 ± 0.40	1.35 ± 0.33	1.06 ± 0.31
HDL-C without MI/CHD (mmol/L)[c]	1.35 ± 0.33	1.40 ± 0.36	1.30 ± 0.40	1.36 ± 0.33	1.07 ± 0.31
HDL-C with MI/CHD (mmol/L)[c]	1.28 ± 0.30	1.22 ± 0.22	1.09 ± 0.29	1.26 ± 0.34	0.96 ± 0.25
MI/CHD %[c]	35.93%	1.52%	3.60%	6.89%	8.96%
Lipid-lowering medication (%)	NA	8.10%	24.30%	15.90%	30.26%
Lipid-lowering medication with MI/CHD (%)[c]	NA	59.4%	77.94%	53.84%	78.11%

HDL-C, age and BMI are presented as mean ± SD

[a]Smokers were defined as current smokers and ever smokers.

[b]missing BMI values were imputed.

[c]The MI/CHD status was determined based on medical records in SCHS while the MI/CHD status in SP2, SCES, SiMES and SINDI was determined based on the responses to the following questions, “Has your doctor ever told you that you have blockage of the arteries to your heart” or “Have you had ever had a heart attack”.

NA: Not available

Table 2

Main and interactive effects of rs2267668 (PPARδ) and rs7191411 (EMP2) SNPs on rank-based inverse normal transformated HDL-C levels

		Discovery		**Meta**	**Replication**				**Meta-analysis**	
		SCHS control	**SCHS case**	**SCHS**	**SCES**	**SP2**	**SiMES**	**SINDI**	**SCES+SP2**	**SCHS+SCES+SP2**
Ethnicity		Chinese	Chinese	Chinese	Chinese	Chinese	Malay	Indian	Chinese	Chinese
N		1,278	715	1,993	1,860	2,088	2,507	2,525	3,948	5,941
MAF	rs2267668	0.27	0.28	0.28	0.29	0.27	0.30	0.15	0.28	0.28
	rs7191411	0.06	0.06	0.06	0.07	0.07	0.07	0.11	0.07	0.07
Model 1 rs2267668 (*PPARδ*)	Beta	−0.02	−0.06	−0.04	−0.02	−0.03	0.02	0.05	−0.03	−0.03
	S.E	0.05	0.07	0.04	0.04	0.04	0.04	0.04	0.03	0.02
	P	0.70	0.33	0.41	0.59	0.44	0.57	0.20	0.38	0.20
Model 2 rs7191411 (*EMP2*)	Beta	0.01	0.09	0.04	0.05	−0.01	−0.01	0.02	0.02	0.03
	S.E	0.08	0.10	0.06	0.06	0.05	0.05	0.04	0.04	0.03
	P	0.93	0.36	0.51	0.33	0.85	0.85	0.58	0.70	0.42
Model 3 rs2267668 (*PPARδ*)	Beta	−0.13	−0.13	−0.13	−0.06	−0.04	0.04	0.08	−0.05	−0.08
	S.E	0.05	0.07	0.04	0.04	0.04	0.04	0.05	0.03	0.02
	P	**0.02**	0.06	**1.00E-03**	0.14	0.27	0.35	0.07	0.08	**1.00E-03**
rs7191411 (*EMP2*)	Beta	−0.38	−0.16	−0.29	−0.09	−0.06	0.05	0.06	−0.08	−0.15
	S.E	0.10	0.13	0.08	0.08	0.08	0.07	0.05	0.06	0.05
	P	**2.73E-04**	0.24	**1.69E-04**	0.25	0.40	0.53	0.22	0.19	**2.00E-03**
rs2267668xrs7191411 (*PPARδ* × *EMP2*)	Beta	0.87	0.54	0.74	0.28	0.11	−0.11	−0.14	0.20	0.35
	S.E	0.16	0.20	0.12	0.11	0.11	0.10	0.10	0.08	0.07
	P	**2.70E-08**	**6.00E-03**	**2.97E-09**	**0.01**	0.31	0.29	0.15	**0.01**	**8.562E-08**

Models 1 & 2: The main effects of *PPARδ* SNP/EMP2 SNP on rank-based inverse normal transformated HDL-C levels were tested in a dominant model with adjustment of age, age^2, gender, imputed BMI/ BMI, principle component (for non-Chinese cohorts), smoking status and lipid-lowering medication status where available.

Model 3: Model 1 + Second SNP + multiplicative term of *PPARδ* SNP × *EMP2* SNP.

Table 3

Genotypic mean HDL-C levels (mean ± SD) of the combined genotypes of rs2267668 (PPARδ) and rs7191411 (EMP2) in the discovery and replication Chinese cohorts

Genotype (rs2267668/rs7191411)	AA/GG	AA/GA	AA/AA	AG/GG	AG/GA	AG/AA	GG/GG	GG/GA	GG/AA
SCHS (Discovery)									
N	908	136	2	699	84	4	137	20	1
HDL-C(mmol/L)	1.34 ± 0.32[ab]	1.27 ± 0.30[cd]	1.19 ± 0.61[e]	1.30 ± 0.32[fg]	1.41 ± 0.33[c]	1.31 ± 0.44[h]	1.27 ± 0.27[ij]	1.56 ± 0.44[adfi]	2.49[bceghj]
SCES + SP2 (Replication)									
N	1,793	258	13	1,327	229	8	283	45	2
HDL-C(mmol/L)	1.36 ± 0.38	1.34 ± 0.35	1.22 ± 0.24	1.34 ± 0.38	1.39 ± 0.40	1.46 ± 0.26	1.33 ± 0.37	1.49 ± 0.40	1.17 ± 0.20
Meta-analysis of SCHS+SCES+SP2 (Discovery + Replication)									
N	2,701	394	15	2026	313	12	420	65	3
HDL-C (mmol/L)	1.35 ± 0.36[a]	1.32 ± 0.34[b]	1.22 ± 0.28	1.33 ± 0.36[c]	1.39 ± 0.38	1.41 ± 0.32	1.31 ± 0.35[d]	1.51 ± 0.41[abcd]	1.61 ± 0.77

Mean value of HDL-C were compared across different genotypes. Significantly different HDL-C between any two group were denoted with the same symbol (Tukey test, $P < 0.05$)