Phosphatidylethanolamine N-methyltransferase gene rs7946 polymorphism plays a role in risk of nonalcoholic fatty liver disease: evidence from meta-analysis

Hwa-Li Tan\textsuperscript{a}, Rosmawati Mohamed\textsuperscript{b}, Zahurin Mohamed\textsuperscript{a} and Shamsul Mohd Zain\textsuperscript{a}

Background Phosphatidylethanolamine N-methyltransferase (PEMT) governs the secretion of hepatic triglycerides in the form of very low-density lipoprotein and has been implicated in nonalcoholic fatty liver disease (NAFLD). Studies on the role of the PEMT rs7946 polymorphism as a genetic modifier of NAFLD have reported inconsistent results. This meta-analysis was carried out to evaluate and summarize the association of PEMT rs7946 with susceptibility to NAFLD.

Methods A comprehensive literature search in Scopus, PubMed, Embase, Science Direct and Google Scholar was performed up to 31 August 2015, followed by data extraction and examination of summary estimates.

Results Six independent studies with a total of 792 NAFLD cases and 2722 controls fulfilled the inclusion criteria. Pooled results indicated that the rs7946 A-allele was associated significantly with an increased risk of NAFLD [odds ratio (OR) 1.55, 95% confidence interval (CI) 1.14–2.11, \( P = 0.005 \)]. A significant association was also found in alternative genetic models of inheritance: dominant, recessive and homozygote (OR 1.62, 95% CI 1.10–2.39, \( P = 0.01 \); OR 1.42, 95% CI 1.12–1.81, \( P = 0.003 \); and OR 1.64, 95% CI 1.18–2.29, \( P = 0.004 \), respectively).

Subgroup analysis by ethnicity indicated a significant association only in the East-Asians in the additive (OR = 2.08, 95% CI 1.12–3.86, \( P = 0.02 \)), recessive (OR = 2.94, 95% CI 1.60–5.37, \( P = 0.0005 \)) and homozygote (OR = 1.86, 95% CI 1.15–3.01, \( P = 0.01 \)) models.

Conclusion This study provides evidence of a significant association between the PEMT rs7946 A-allele and a risk of NAFLD, with the effect being more prominent in East-Asians, but not in non-Asians. Pharmacogenetics and Genomics 00:000–000 Copyright © 2015 Wolters Kluwer Health, Inc. All rights reserved.

Keywords: fatty liver, genetic variation, meta-analysis, nonalcoholic fatty liver disease, PEMT, polymorphism, single nucleotide polymorphism

Introduction Nonalcoholic fatty liver disease (NAFLD) encompasses a full pathological spectrum of progressive liver disease from simple steatosis (simple fatty infiltration of liver) to nonalcoholic steatohepatitis (NASH) (inflammation and/or fibrosis in addition to fat accumulation) to cirrhosis [1]. NAFLD occurs in individuals with no significant alcohol consumption and its incidence increases in parallel with the prevalence of metabolic syndrome [2]. As a rapidly emerging disease, the prevalence rate of NAFLD ∼ 11–45% in the general population, with an alarming rate of greater than 50% in obese children and adolescents [3]. Corresponding to an increase in sedentary lifestyle and change in dietary patterns, the prevalence of NAFLD has become a rising concern and considered the most common chronic liver disease in developed and developing countries [4].

Because of the higher risk of liver-related morbidity and mortality in patients with fatty liver compared with the general population, NAFLD has gained clinical and research priority [5,6]. Being a complex metabolic disease, the exact aetiology of NAFLD has not been fully elucidated. Studies on familial aggregation and inter-ethnic differences in susceptibility carried out in the last decades led to interest in the heritable components of NAFLD [7,8]. In line with this, several meta-analyses on gene polymorphisms and risk of NAFLD have been reported in various cohorts [9–15].

The hallmark of NAFLD is the accumulation of triglycerides in the liver that occurs as a result of permanent imbalance between fatty acid influx, utilization and secretion of very low-density lipoproteins (VLDL) [16, 17]. In hepatic tissues, phosphatidylethanolamine N-methyltransferase (PEMT) catalyses the biosynthesis...
of phosphatidylcholine (PC), which is required in the packaging and export of triglycerides in the form of VLDL [18,19]. Decreased PEMT activity reduces PC availability and thus causes fat droplets to accumulate in the cytosol of hepatocytes [20–22]. Single nucleotide polymorphisms (SNPs) in the PEMT gene have been linked to hepatic PEMT activity. One of the notable SNP is rs7946, a nonsynonymous variant characterized by the G to A substitution in the exon 8 of PEMT, which results in the amino acid replacement of valine with methionine (V175M) [23]. It is therefore hypothesized that PEMT rs7946 is associated with the risk of NAFLD.

Ethnic disparities with respect to the genetic modifiers of NAFLD remain a challenging issue to date. The role of the PEMT rs7946 variant in the predisposition of NAFLD was first suggested in a mixed population in the study by Song and colleagues, and was later supported by several studies in the East-Asians [23–26]. However, the role of rs7946 SNP in NAFLD remains controversial because of conflicting reports in different populations and ethnic variations [27,28]. Large and unbiased genetic epidemiology studies of PEMT are crucial to confirm its role in NAFLD. The aim of this study is to investigate the association of PEMT rs7946 with susceptibility to NAFLD through a meta-analysis incorporating all relevant studies.

Methods

Search strategy

In keeping with the criteria established by the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) [29], we performed a systematic literature search in Scopus, PubMed, Embase, Science Direct and Google Scholar, with the last update being on 31 August 2015. The following keywords were used in the searches: (‘Phosphatidyl ethanolamine methyltransferase’ or ‘Phosphatidylethanolamine N-methyltransferase’ or ‘PEMT’) and (‘fatty liver’ or ‘steatohepatitis’ or ‘NAFLD’ or ‘NASH’) and (‘polymorphism’ or ‘mutation’ or ‘genotype’ or ‘allele’ or ‘variation’ or ‘variant’). Additional potentially related studies including review articles and letter to editors related to this topic were identified by a hand search of the references in selected literatures. Abstracts of presentations at international meetings (American Association for the Study of Liver Disease and European Association for the Study of the Liver) were screened from 2012 to 2015.

Inclusion and exclusion criteria

Studies were included on the basis of the following criteria: (i) studies that evaluated the association between the PEMT rs7946 and NAFLD; (ii) case–control studies; (iii) studies that had detailed the genotype frequency of cases and controls or could be calculated from the paper text; (iv) included the underlying NAFLD as the outcome of the study; and (v) articles published in Chinese and English. The major exclusion criteria were as follows: (i) case-only studies, case reports and review articles; (ii) studies without the raw data of the PEMT rs7946 genotype or insufficient data for extraction; (iii) assessed other PEMT SNPs, but not rs7946; (iv) only included patients with metabolic syndromes such as obesity or dyslipidaemia without assessment for NAFLD; and (v) duplicated studies.

Data extraction

The following information was collected from each eligible publication: author’s name, year of publication, study design, country of origin, ethnicity, sample size, diagnostic methods, genotyping methods and PEMT genotypes. All the data of the eligible studies were extracted on the basis of the inclusion and exclusion criteria by two investigators independently. The data were compared and all disagreements were resolved by consensus. In the event of missing data, the corresponding authors were contacted as needed. The genotype data that were not available in published articles were calculated on the basis of the effective allele frequency and validated with the original author. The quality of the studies included was assessed according to the Newcastle–Ottawa Scale (NOS) [30] on a scale of 0–9, taking into account three aspects: (i) the selection of the study groups; (ii) the comparability of the groups; and (iii) the ascertainment of either the exposure or the outcome of interest for case–control or cohort studies, respectively.

Data analysis

The Hardy–Weinberg equilibrium of the genotypes distribution in each study was assessed using the \( \chi^2 \)-test. The pooled odds ratio (OR) and 95% confidence interval (95% CI) were calculated to estimate the association of the PEMT polymorphism with susceptibility to NAFLD for each genetic model of inheritance: additive (A vs. G), homozygote (AA vs. GG), heterozygote (GA vs. GG), dominant (GA/AA vs. GG) and recessive (AA vs. GA/GG) models, where \( P \) less than 0.05 indicates statistical significance.

Studies with mixed ethnicities such as in Romeo et al. [27], were subjected to ethnic stratification into separate entries before statistical analysis because of an effective allele frequency difference between different ethnicities. The heterogeneity assumption was evaluated using a \( \chi^2 \)-test-based \( Q \)-statistic. Heterogeneity effect was quantified by the inconsistency index \( I^2 \) metric test. When the value for the Q-test (\( P < 0.10 \)) or \( I^2 \) greater than 50% suggested heterogeneity across studies, the ORs were pooled by the random-effect model; otherwise, a fixed-effect model was adopted [31]. Analysis of sensitivity was carried out by individually removing the included studies to evaluate the stability of the results. Furthermore, potential publication bias was investigated using Begg’s
funnel plot and Egger’s regression test [32,33]. To address pronounced publication bias, the Trim and Fill method [34] was used (which calculates the estimates with adjustment of the bias by imputing data from potential missing studies). To minimize the influences of disparities in the studies, the study populations were stratified into East-Asians (n = 4) and non-Asians (n = 4). A subgroup comprising only of NASH patients (n = 2) was also analysed. Although the utilization of ultrasonography and magnetic resonance spectroscopy in the other four studies enabled detection of the presence of hepatic steatosis, they could not distinguish NASH from NAFLD [25–28]. Therefore, analysis of non-NASH subgroup could not be carried out. All analyses were carried out using the Cochrane Collaboration RevMan 5.3 and STATA package, version 12.0 (StataCorporation, College Station, Texas, USA).

Results

Study characteristics

A total of 19 potentially relevant literatures were identified for inclusion in the review, including a systematic search of electronic medical database and a hand search of cited literature. After adjusting for duplicates, 18 studies remained. Following screening, seven studies were excluded, and the full text of the remaining 11 citations was examined in detail. On the basis of the selection criteria, six eligible studies including a Chinese-written literature were included. The flow chart of the selection process is summarized in Fig. 1. The reporting of this meta-analysis was presented in accordance with the PRISMA statement for which the checklist was provided in Supplemental digital content 1, http://links.lww.com/FPC/A938.

The study size ranged from 87 to 2222, with a total of 792 NAFLD cases and 2722 controls, for the assessment of the PEMP rs7946 G > A polymorphism. A mixture of ethnic groups was assessed in which the majority were East-Asians, followed by Caucasians, Hispanics and Blacks. The detailed characteristics including genotypic distribution were extracted successfully from the study articles and are shown in Table 1. Liver biopsy, followed by histological assessment of NAFLD was performed in two of the studies [23,24], whereas the other four studies used imaging modalities in the diagnosis [25–28]. All of the studies were carried out in adults, except the study of Song et al. [23], which included patients who ranged in age from 5 to 77 years. Four studies were hospital-based case–control studies [23,24,26,28] and the remaining two were population-based studies [25,27]. All studies were considered high quality with an NOS score of at least 7, except Song et al. [23], which was of moderate quality (Supplemental digital content 2, http://links.lww.com/FPC/A939, which shows the assessment score for study quality).

Quantitative data synthesis

The different genetic models for the respective independent studies are tabulated in Supplemental digital content 3, http://links.lww.com/FPC/A940. A summary of the association between the PEMP rs7946 G > A polymorphism and susceptibility to NAFLD is presented in Fig. 2 and Table 2. Overall, evidence of an increased risk of NAFLD was found in all genetic models of inheritance: additive (OR = 1.55, 95% CI = 1.14–2.11, P = 0.005), dominant (OR = 1.62, 95% CI = 1.10–2.39, P = 0.01), recessive (OR = 1.42, 95% CI = 1.12–1.81, P = 0.003) and homozygote (OR = 1.64, 95% CI = 1.18–2.29, P = 0.004), with the exception of a heterozygote model. When we collapsed the studies on the basis of biopsy-proven NASH patients [23,24], the PEMP rs7946 A-allele was found to be associated with an increased risk of NASH in the additive (OR = 3.76, 95% CI = 2.14–6.59, P < 0.00001), dominant (OR = 3.59, 95% CI = 1.61–7.98, P = 0.002) and homozygote (OR = 8.94, 95% CI = 2.32–34.39, P = 0.001) models. Similarly, other studies [25–28] that utilized a medical imaging technique showed a significantly increased risk of NAFLD in the additive model (OR = 1.27, 95% CI = 1.01–1.60, P = 0.04), but not in the other genetic models.

In the subgroup analysis, we defined ethnicity as East-Asian or non-Asian; a significant association was found in the East-Asians in additive (OR = 2.08, 95% CI = 1.12–3.86, P = 0.02), dominant (OR = 2.94, 95% CI = 1.60–5.37, P = 0.0005) and homozygote (OR = 1.86, 95% CI = 1.15–3.01, P = 0.01) models and borderline significance in the dominant model (OR = 1.95, 95% CI = 1.01–3.79, P = 0.05). However, no clear evidence of association was observed in the non-Asians (P > 0.05) (Table 2).

Test of heterogeneity and sensitivity

Significant heterogeneity was detected in the pooled estimates when different comparisons were made. As shown in Table 2, the P values and ORs were calculated on the basis of a random-effect model when significant heterogeneity was observed. As the study weight differs from one population to another, thereby introducing the probability of effect modification, sensitivity analysis was carried out to examine the influence of the individual study on the pooled estimates by excluding individual studies one by one. The results of sensitivity analysis suggested that no single study could influence the overall estimates, indicating statistically robust results.

Publication bias

On primary visual inspection, the asymmetry plot existed suggesting the presence of publication bias (Supplemental digital content 4, http://links.lww.com/FPC/A941, which shows a funnel plot of the variant under additive model). The Trim and Fill method [34] was used to adjust the results in the comparisons. The
corresponding pooled ORs were not significantly altered in the comparisons.

**Discussion**

In an attempt to elucidate the association of *PEMT* rs7946 with the risk of NAFLD, a meta-analysis that includes six case–control studies was carried out. The *PEMT* rs7946 nonsynonymous SNP was first described by Song et al. [23] as a risk factor of NAFLD in a small population of predominantly Caucasians. The recruitment of controls, although strict, was not performed from the same community and thus contributed toward a lower NOS score. Subsequently, a larger study including three ethnicities (Caucasians, Hispanics and African Americans) by Romeo et al. [27] failed to replicate the association despite showing a relatively similar risk allele frequency in their White patients (80%) as reported in the study by Song and colleagues (79%). Four other studies were carried out in East-Asian populations and reported an increased risk of NAFLD in individuals with the *PEMT* rs7946 A-allele, except for the Korean populations [24–26, 28]. The interpretation of results is challenging and contradictory, given the observed heterogeneity in allelic frequency between different populations.

In this meta-analysis, to ensure a more homogenous evaluation of study outcome, the study by Romeo and colleagues was stratified on the basis of ethnicity into Caucasians, Hispanics and African Americans with respective risk allele frequencies and ORs in the analysis.
The *PEMT* rs7946 was found to be associated with NAFLD and NASH, with a pooled effect of 1.27- and 3.76-fold increased risk, respectively, when the A-allele was compared with the G-allele. PEMT plays a pivotal role in catalysing methylation of phosphatidylethanolamine (PE) into PC, which is required for hepatic secretion of triglycerides in the form of VLDL [18,35]. The *PEMT* rs7946 variant causes valine to methionine amino acid substitution (V175M), and thus results in an inadequate level of hepatic PC, which impairs the rate of fat removal [23]. Aberrant VLDL-mediated secretion of triglycerides is a central mechanism of NAFLD and this supports the notion that the *PEMT* rs7946 polymorphism increases the risk of NAFLD [36].

Subgroup analysis carried out by categorizing the populations into East-Asians and non-Asians suggested that the effective A-allele of rs7946 seems to lead to an increased risk in the East-Asians. However, this was not the case in non-Asians; the effective A-allele was present in higher frequencies in healthy controls among Caucasians (73%), Hispanics (52%) and African Americans (38%) [27]. One of the possible explanations for this is that under normal circumstances, the rate of triglyceride synthesis is slow enough for the packaging and export of fat to be sustained by the mutant PEMT activity [37]. Only during rapid triglyceride production such as excessive calorie intake would reduced PEMT activity be a limiting factor, thus resulting in hepatic steatosis. Hence, the *PEMT* rs7946 A-allele may be necessary, but not sufficient to cause fatty liver [37]. This may also justify why the most consistent and prominent effect of the *PEMT* rs7946 variant was observed in the homozygote model, whereby two mutant alleles are required to cause a significant alteration in the disease phenotype.

In addition, NAFLD is a multifactorial disease whereby the interplay between host genetic and environmental factors is of concern. One of the relevant dietary factors is choline, which is a major source of PC: when dietary choline is restricted, all hepatic PC syntheses would rely on the PEMT activity [38], whereas under normal conditions, the PEMT pathway accounts for only 20–30% of PC synthesized [19]. *PEMT* knockout mice do not show PEMT activity and depend solely on dietary choline intake. A choline-deficient diet exacerbated liver injury in *PEMT* knockout mice, whereby liver failure developed in 3 days in addition to a 50% reduction in hepatic PC concentration compared with wild-type mice on a choline-deficient diet [39]. The lethality of choline-deprived *PEMT* knockout mice is because of the primary excretion of hepatic PC by biliary secretion as mice with defective PC flippase in addition to *PEMT* knockout have impaired PC secretion of bile and do not show any liver injury [40]. Another study carried out on high-fat diet-induced NAFLD in *PEMT*-knocked out mice found that mice with choline supplementation increased
the hepatic PC/PE ratio and decreased inflammation, suggesting the role of diet in alleviating NAFLD despite the presence of a genetic predisposition [41]. In humans, choline supplementation was found to ameliorate hepatic steatosis that is associated with parenteral nutrition [42]. The main food sources of choline include red meat, eggs, poultry and milk, which are predominant in the Western diet [43]. In comparison, the total choline intake is much lower in Asians compared with Caucasians and African Americans [44]. Therefore, the effect of *PEMT* rs7946 is not prominent in non-Asian populations because of the higher total choline intake through the diet. To improve the impact and the significance of *PEMT* rs7946, future studies should attempt to investigate other SNPs that are in close proximity to the *PEMT* rs7946, and also the environmental effect of a choline diet and other SNPs that may affect the biliary secretion of PC. This may lead to elucidation of the environmental and genetic interactions in the development of NAFLD and thus provide insights into a possible diet that can ameliorate hepatic injury. It is also important to investigate the SNPs that are in close proximity to the *PEMT* rs7946.

This meta-analysis has several limitations. First, the number of published studies, especially in the non-Asian population, was not sufficiently large for a comprehensive analysis. There were only two independent studies on non-Asian populations, comprising predominantly Caucasians, Hispanics and African Americans [23,27]. Despite limited numbers of studies, the pooled estimates are not affected by individual study and other factors, hence providing confidence on the results. Second, the results in this meta-analysis are based on unadjusted estimates, and the lack of such information (age, sex, racial and geographical factors) in the studies limits the confidence in the results. However, the statistical analysis included a sensitivity analysis, which included a fixed effect model that did not alter the results. The sensitivity analysis is important as it increases the reliability of the results.

Table 2 Stratified analysis of the *PEMT* rs7946 and NAFLD risk

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<td>East-Asian</td>
<td>4</td>
<td>1.95 [1.01–3.79]</td>
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<td>Non-Asian</td>
<td>4</td>
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<td>Total</td>
<td>8</td>
<td>1.62 [1.10–2.39]</td>
<td>0.01*</td>
<td>1.42 [1.12–1.81]</td>
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Dominant model, GA + AA versus GG; recessive model, AA versus GA + GG; homozygote model, AA versus GG; heterozygote model, GA versus GG. CI, confidence interval; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio.

* A random-effects model was used because of heterogeneity in studies.
family history, dietary pattern and other risk factors) can have an impact on the actual values. Third, only two out of the six studies included had performed liver biopsy, which would have enabled the distinction between NASH from non-NASH samples. Despite the difference in diagnosis methods, the clinical diagnosis of NAFLD patients was assessed thoroughly and liver diseases of other aetiologies such as viral hepatitis, drug-induced hepatitis, autoimmune disease and primary biliary cirrhosis were excluded. The association of risk A-allele was more prominent in studies involving liver biopsy compared with medical imaging, which can be explained by disparities in the severity of NAFLD; only NASH patients were included in the former studies and the latter recruited patients with NAFLD of various severities. Nonetheless, this meta-analysis has several strengths. This is the first meta-analysis to comprehensively assess and show a significant association between PEMT rs7946 and the risk of NAFLD. Second, we explored the relationship between this variant and NAFLD in different genetic models of inheritance. Third, despite challenges in the variability of diagnostic and genotyping methods and the presence of ethnic differences, the confidence of the results is derived from the observed analytical measurements. All of the East-Asian studies are within the insulin resistance (HOMA-IR) cut-off values. HOMA-IR measurement is, however, not available for the non-Asian studies. NAFLD patients from all the studies have a mean BMI up to 30 kg/m² and the glucose levels are within the normal range, except a borderline value found in the study by Zhou et al. [25]. Finally, the results of this meta-analysis showed a distinct risk predisposition in different populations, suggesting the possible interaction of PEMT and dietary pattern in the pathogenesis of NAFLD.

In summary, the data from the present study indicate that the PEMT rs7946 may act as a genetic modifier of NAFLD, particularly in East-Asians. It is crucial that larger studies with a standardized diagnostic method and well-matched controls be attempted in the future to clarify the association of PEMT rs7946 with the risk of NAFLD.

Acknowledgements
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Conflicts of interest
There are no conflicts of interest.

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