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Role of CYP2C9 polymorphism in phenytoin-related metabolic abnormalities and subclinical atherosclerosis in young adult epileptic patients

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ABSTRACT

Purpose: We investigated the influence of the CYP2C9 polymorphism on the lipid profile, insulin resistance, and subclinical atherosclerosis in young epileptic patients.
Methods: We performed a cross-sectional study to evaluate the association between CYP2C9 polymorphism and lipid profile, glucose homeostasis, and subclinical atherosclerosis in young epileptic patients via the ankle brachial index.
Results: The frequencies of CYP2C9*1 (CYP2C9 wild type gene) and CYP2C9*3 (CYP2C9 polymorphism gene) were 75% and 25%, respectively. The mean serum total triglyceride and LDL levels were significantly higher in the wild type gene subjects than in the CYP 2C9 polymorphism gene subjects. Also, the CYP 2C9 polymorphism had marginally significant lower mean serum HDL levels than the wild type gene subjects. No patients with CYP 2C9 polymorphism gene had elevated fasting blood sugar, and insulin resistance was found in only 10 of the 75 subjects. The mean ABI was statistically significantly lower in the wild type subjects than in the CYP2C9 polymorphism gene subjects.
Conclusion: Our study indicates that young epileptic patients with the CYP2C9 polymorphism gene have a low risk of subclinical atherosclerosis.

1. Introduction

The influence of antiepileptic drug (AED) therapy on the development of atherosclerosis has been the subject of controversy, although recent evidence indicates that prolonged antiepileptic treatment might modify some vascular risk factors.[1] The existing data on the effect of phenytoin (PHT) on atherosclerosis are contradictory. There is some evidence that PHT may contribute to protecting some patients form atherosclerosis by increasing the concentration of high density lipoproteinemia cholesterol[2] and atherogenic cholesterol fractions.[3] However, a recent study from Thailand showed PHT inhibited the secretion of insulin and decreased the response of plasma insulin to glucose, a condition known as insulin resistance,[4] which can induce atherosclerosis. However, both apparent positive and negative effects of PHT show individual differences, and the factors that cause such individual differences remain unclear. An experimental study examining the results of PHT treatment on atherosclerosis susceptibility and lipid profile in mice found that PHT treatment had a protective effect, and that the atherosclerosis prevention was not concomitant with a consistent increase in HDL or any other protective change in the lipid profile.[5]

To date, atherosclerosis remains a complex multifactorial disorder that is thought to result from an interaction between the genetic make-up of an individual and various environmental factors. Cytochrome P450 is partly responsible for the biosynthesis of endogenous vasoregulators.[6] In addition, CYP2C9 also generates significant amounts of oxygen-derived free radicals.[7] The other mechanism of PHT-induced atherosclerosis is via insulin resistance and increased total cholesterol, total triglyceride, and LDL-C. Currently, no studies are available on the impact of CYP2C9 mutation alleles on metabolic abnormalities and subclinical atherosclerosis of young epileptic patients who are taking PHT as monotherapy. We therefore investigated the influence of the CYP2C9*2 and CYP2C9*3 variants on the lipid profile, insulin resistance, and subclinical atherosclerosis in young epileptic patients with who were receiving PHT monotherapy in Thailand.

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1.1. Subjects and methods

This was a single-center, university hospital-based, cross-sectional study conducted from October 2010 to May 2012. We recruited ambulatory young Thai adults with epilepsy who had been treated with phenytoin monotherapy for at least 2 years. All of our patients had focal onset epilepsy. The inclusion criteria were: a Thai national with epilepsy; age 20–40 years, stable dosage of PHT (during the previous 6 months), no chronic medical illness other than epilepsy, taking no medication except an antiepileptic, with an active daily life (could perform activities of daily living without assistance), not drinking alcohol or smoking, and no family history of diabetes. Patients who had previously taken an AED but had stopped at least 3 years prior to the beginning of this study were allowed. Because at our institution we routinely give folic acid to epileptic patients, patients taking a stable dosage of folic acid (5 mg/d) for at least 1 year were also accepted. Exclusion criteria were: pregnancy; having a significant medical disorder other than epilepsy known to affect lipid metabolism or glucose homeostasis, alcohol drinking, and/or smoking in the past 5 years.

The Ethics Review Committee of the Faculty of Medicine, Prince of Songkla University, approved the study and informed consent was sought and obtained from all patients.

Each subject completed a questionnaire covering basic demographics such as sex and age, and medical details such as age at onset of epilepsy, duration of treatment, and current and past medication usage. Weight and height were also measured and the body mass index was calculated [body weight (kg)/height^2 (m)].

1.2. Laboratory procedures

Baseline control data were obtained in the morning after a 10-h overnight fasting period. The serum glucose level was determined by enzymatic hexokinase (Modular P800; Roche Diagnostics, Germany) and the serum insulin level by electrochemiluminescence immunoassay [ECLIA] (Modular E170; Roche Diagnostics, Germany). The sensitivity of the assay was 0.200 mU/L, and the coefficients of variation were 1.38% and 0.87% for intra-assay, and 4.20% and 3.97% for interassay, respectively (intra-assay: $n = 20$, mean = 24.06 mU/L, SD $\pm$ 0.33 and mean = 80.05 mU/L, SD $\pm$ 0.70; interassay: $n = 20$, mean = 22.70 mU/L, SD $\pm$ 0.95 and mean = 79.26 mU/L, SD $\pm$ 3.15).

Using the fasting glucose and insulin levels, we calculated the homeostasis model assessment (HOMA) index for each patient, using the following equation:

$$\text{HOMA-index} = \frac{\text{fasting glucose (mmol/L} \times \text{fasting insulin (mIU/L))}}{22.5}$$

HOMA-IR values have been shown to correlate well with values obtained using the "gold standard" clamp technique.[8] Patients above the threshold of $\geq$1.73 were classified as having insulin resistance.[9] Trough serum levels for AEDs were examined.

1.3. Diagnosis of metabolic syndrome

The criteria of metabolic syndrome require at least three of the following conditions: (1) obesity, (2) hypertriglyceridemia (at least 150 mg/dL), low high-density lipoprotein (HDL) cholesterol (<40 mg/dL in men and <50 mg/dL in woman), and (3) hypertension (at least 130/85 mm Hg, and (4) impaired glucose tolerance, according to the Adult Treatment Panel III.[10]

1.4. ABI measurement

Standardized ABI measurements were performed according to the AHA recommendations for the evaluation of peripheral artery disease (PAD) by a physician with experience in ABI recording. ABI was measured using a handheld 8 MHz handheld Doppler device (MiniDop ES-100VX, Hadeco Inc, Japan). Systolic pressure was measured both in the dorsalis pedis and in the posterior tibial artery. The higher of these pressures was divided by the higher of the two brachial systolic pressures to calculate the ABI. An ABI $\leq$ 0.90 in either leg was considered as evidence of asymptomatic PAD, and an ABI >0.90 was considered as normal.[11]

1.5. CYP2C9 genotyping

Genomic DNA was isolated from leukocyte nucleids using a QIAamp DNA Mini Kit (QIAGEN). Realtime HRM PCR (realtime polymerase chain reaction high resolution melting) was performed in a strip tube with a reaction volume of 20 μL, containing 2 μL of SsoFast Evagreen Supermix "BioRad" (ready to use reactionmix), 0.15 μM of the forward and reverse primers for CYP2C9*2 and CYP2C9*3, and 100 ng/μL of genomic DNA. The sequences of forward and reverse primers used were respectively 5′TACAAATACAATGAAAATATCATG3′ and 5′ CTAACAACCAGA CTCATAATG 3′ for CYP2C9*2 (Arg144Cys) genotype and 5′ AATAATAATATGCACGAGGTCCAGAGGTAC 3′ and 5′ GATACTAT-GAATTTGGGACTT C 3′ for CYP2C9*3 (Ile359Leu) genotype (these primers were described by Ramasamy et al.[12] and synthesized by BioDesign Co., Ltd.).

Realtime PCR amplification to detect CYP2C9*2 and/or CYP2C9*3 was performed using CFX 96 Connect Realtime-PCR (Bio-Rad Laboratories USA) with an initial denaturation at 95.0 °C for 3 min, followed by 40 cycles of denaturation at 95.0 °C for 10 s, annealing at 59.0 °C for 15 s, and final extension at 95.0 °C for 10 s. A melt curve was plotted from 73.0 °C to 85.0 °C, with an increment of 0.2 °C every 10 s. The analysis of melt curves used a high-resolution melt curve (HRM) method on Precision Melt Analysis software version 1.1 (Bio-Rad Laboratories USA). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) used the same primer sets as the Realtime PCR. PCR was performed at a reaction volume of 20 μL, consisting of 2 μL of Top Taq Master Mix ready-to-use reaction mix (QIAGEN), 0.2 μM each of the forward and reverse primers for CYP2C9*2 and CYP2C9*3, and 100 ng/μl of genomic DNA. PCR was performed in a S1000-PCR thermal cycler (Bio-Rad Laboratories USA) with an initial denaturation at 94.0 °C for 4 min, followed by 40 cycles of denaturation at 95.0 °C for 30 s, annealing at 56.0 °C for 30 s, extension at 72.0 °C for 45 s and final extension at 72.0 °C for 10 min. An 8 μL alliquot from each PCR product was digested with 5 U of restriction enzymes AvaII (Promega) and 5 U of Kpn I (Takara), for CYP2C9*2 and CYP2C9*3, respectively. Incubation was done at 37 °C overnight. Detection of the digested product was performed with 12% polyacrylamide gel electrophoresis, using 120 V for 2 h 30 min. The PCR-RFLP results were randomly selected to be confirmed by direct nucleotide sequencing on an ABI 3130 Genetic Analyzer with 3130 Collection Software.

2. Statistical analysis

The characteristics of the study patients were described in terms of mean and standard deviation for continuous variables, and number and percentage for categorical variables. Comparisons of continuous variables between the two subgroups of subjects were made using a two-tailed *t*-test. Correlations between serum level of PHT and fasting glucose, fasting insulin, and lipid profile parameters in the CYP2C9 gene group were explored using Pearson product moment correlation coefficient.

Table 1
Demographic and other data of study patients.

Variable	Wild type gene ($n = 56$)	CYP2C9 polymorphism ($n = 19$)
Average age (years; range)	29.93 (20–40)	30.42 (20–40)
Sex (male:female)	39:17	12:7
Average duration of AED use (years; range)	3.85 (2–5.7)	3.47 (2–5.5)
Type of seizure (n)		
Focal onset		
Simple with or without generalized seizure complex	40	14
Partial with or without generalized seizure	16	5
Etiology		
Unknown	51	17
Structural		
Head injury	3	1
Central nervous system infection	2	1
Through serum PHT level (mean; range)	16 (10–23)	19 (17–23)
Seizure control		
Seizure free[a]	42	14
Well-controlled[b]	14	5

[a] Defined as seizure free for >24 months under AED therapy.
[b] Defined as seizure free for >12 months under AED therapy.

Statistical analyses were performed using Stata version 7.0 (Stata Statistical Software: Level 7.0 College Station, TX, USA).

3. Results

For the present study, we recruited 75 young epileptic patients who were taking PHT monotherapy, whose demographic characteristics and basic laboratory data are shown in Table 1. Ninety one percent had a diagnosis of unknown epilepsy, 5% of head injury, and 4% of central nervous system infection (Table 1). A majority (75%) were seizure free for more than 24 months. There were 51 males and 24 females, with a mean age (±SD) of 30.1 ± 5.6years (range: 20–40 years). The mean duration of treatment (±SD) with antiepileptic drug use for the overall group at the time of the study was 3.8 ± 0.99 years (range: 2–5.7 years). The mean (±SD) serum level of PHT was 16.8 ± 3.2 (range 10–23) mg/L. About 24% (18 out of 75) were found to be CYP2C9*3 heterozygous carriers (wt/mt), and 1.3% (1 out of 75) were CYP2C9*3 homozygous (mt/mt), which is characterized by poor PHT metabolism. No CYP2C9*2 polymorphism were identified.

We then classified the patients into 2 groups: (1) the CYP2C9 polymorphism gene group, which included patients who had homozygous or heterozygous CYP2C9*3; and (2) the wild type gene of CYP2C9 group, which included those identified as wild type CYP2C9*1. The CYP2C9 polymorphism group included 19 members, 12 males and 7 females, with a mean (±SD) age of 30.4 (±5.6) years (range 20–40 years). There were no significant differences between the CYP2C9 polymorphism group and the wild type gene group in sex or age. The mean (±SD) duration of treatment was not significantly different between groups; those in the CYP2C9 polymorphism group had been treated for an average of 3.47 ± 1.0 years (range: 2–5.5 years), while the wild type gene group had been treated for an average of 3.85 ± 0.98 years (range: 2–5.7 years) ($p = 0.15$). The other basic demographics of both groups are shown in Table 1; there were no significant differences in any variables between the groups. However, the CYP2C9 polymorphism group had higher serum levels of phenytoin, compared with the wild type gene group ($p < 0.01$).

The individual vascular risk factors in both groups are summarized in Table 2. The mean (±SD) serum total cholesterol

Table 2
Vascular risk factors and ABI in study patients.

Characteristic	CYP 2C9 genotype				p value
	Wild type ($n = 56$)		CYP2C9 polymorphism ($n = 19$)		
	Mean ± SD	95% CI	Mean ± SD	95% CI	
Average age, SD (years)	29.2 ± 5.3	28.5–31.3	30.4 ± 5.6	27.2–33.6	0.74
Sex					0.92
Male (n)	39		12		
Female (n)	17		7		
Duration of treatment (years)	3.85 ± 0.98	3.59–4.11	3.47 ± 1.0	2.99–3.96	0.15
Serum phenytoin level (mg/L)	15.9 ± 3.1	15–17	19 ± 1.9	18–20	<0.01
BMI (kg/m^2)	24.4 ± 2.5	23.7–25.1	24.5 ± 2.9	23.1–26	0.84
Fasting blood glucose (mmol/L)	93.9 ± 7.7	91.8–95.9	97.8 ± 5.8	95.0–100.6	0.04
Systolic blood pressure (mm Hg)	131.4 ± 7.8	129.3–133.5	132.5 ± 6.3	129.4–135.5	0.58
Diastolic blood pressure (mm Hg)	81.0 ± 6.8	79.2–82.9	82.7 ± 5.7	80.0–85.5	0.33
Fasting blood insulin (mIU/L)	6.4 ± 1.1	6.1–6.7	7.2 ± 2.2	6.1–8.2	0.04
Total triglycerides (mg/dL)	147.9 ± 35.5	138.4–157.4	129.1 ± 16.9	121.0–137.3	0.03
Total cholesterol (mg/dL)	209.8 ± 37.8	199.7–220.0	186.9 ± 40.3	167.5–206.4	0.03
LDL cholesterol (mg/dL)	134.5 ± 26.1	127.5–141.5	116.8 ± 35.3	99.8–133.9	0.02
HDL cholesterol (mg/dL)	65.4 ± 5.1	64.1–66.8	60.9 ± 15.1	53.6–68.1	0.05
HOMA	1.48 ± 0.37	1.40–1.55	1.63 ± 0.59	1.35–1.92	0.12
ABI					
Left leg	0.94 ± 0.08	0.92–0.96	0.98 ± 0.06	0.95–1.00	0.03
Right leg	0.92 ± 0.08	0.89–0.94	0.96 ± 0.04	0.94–0.98	0.04

BMI, body mass index; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; HOMA, homeostasis model assessment. ABI, ankle brachial index.

Table 3
Prevalence of individual components of vascular risk factors by sex in patients with the CYP 2C9 genotype polymorphism.

Variable	Wild type ($n = 56$)	CYP 2C9 polymorphism ($n = 19$)
Glucose homeostasis		
Fasting blood sugar (≥110)	2	0
Abnormal HOMA (≥1.73)	11	10
Lipid profile		
Total cholesterol (≥200 mg/dL)	35	8
Total triglyceride (≥150 mg/dL)	31	4
LDL (≥130 mg/dL)	16	4
HDL (<40 mg/dL in men, <50 mg/dL))	0	1
Overweight (BMI > 23)	39	12

BMI, body mass index; LDL, low-density lipoprotein cholesterol; HDL, high-density lipoprotein cholesterol; HOMA, homeostasis model assessment.

levels were significantly higher ($p = 0.03$) in the wild type gene group than in the CYP 2C9 polymorphism group (8 ± 37.8 vs. 186.9 ± 40.3). The mean (±SD) serum total triglyceride and LDL levels were also significantly higher in the wild type gene group than in the CYP 2C9 polymorphism group (147.9 ± 35.5 vs. 129.1 ± 16.9, $p = 0.03$ and 134.5 ± 26.1 vs. 116.8 ± 35.3, $p = 0.02$ respectively). The wild type group had marginally significantly ($p = 0.05$) higher mean (±SD) serum HDL levels than the CYP2C9 polymorphism group (65.4 ± 5.1 vs. 60.9 ± 15.1). Table 3 shows the prevalence of individual risk factors of atherosclerosis by CYP genotype. Overall, elevated fasting glucose (>110 mg/dL) was identified in 2 (2.6%) patients, and insulin resistance (HOMA ≥ 1.73) in 21 (28%) patients. High total concentrations of cholesterol (≥200 mg/dL) were noted in 43 (57.3%) patients. Thirty-five patients (46.7%) had high serum triglyceride concentrations (≥150 mg/dL), 20 (26.7% had high LDL-C concentration (≥130 mg/dL), and 1 (1.3% had low HDL concentrations (<40 mg/dL in men, < 50 mg/dL (<40 mg/dL in men, <50 mg/dL)). In patients with CYP2C9 polymorphism, no patient had elevated fasting blood sugar (>110 mg/dL). Insulin resistance (HOMA ≥ 1.73) was found in 10 of the 19 patients Also, high total concentrations of cholesterol (≥200 mg/dL) were noted in 8 of the 19 patients, high serum triglyceride concentrations (≥150 mg/dL) in 4 of 19 patients, high LDL-C concentrations (≥130 mg/dL) in 4 of 19 patients, and low HDL levels in 1 of 19 patients (Table 3). The mean systolic and diastolic blood pressure levels were not statistically significantly different between the groups ($p = 0.58$ and $p = 0.33$ respectively).

The mean (±SD) ABI was statistically significantly lower in the wild type gene group than in the CYP 2C9 polymorphism group in both legs (left leg 0.94 ± 0.08 vs. 0.98 ± 0.06, $p = 0.03$; right leg 0.92 ± 0.08 vs. 0.96 ± 0.04, $p = 0.04$). Overall, the results of the study showed that ABI was not related to age, gender, serum PHT level, BMI and duration of AED therapy. Also, the fasting blood level was not associated with serum PHT.

4. Discussion

To our knowledge, this is the first study to investigate the effect of CYP2C9 polymorphism on insulin resistance, lipid profile, and subclinical atherosclerosis in epileptic patients taking PHT. The study found that epileptic patients who had the CYP2C9 polymorphism had lower total triglyceride, total cholesterol, LDL cholesterol, and HDL cholesterol levels than epileptic patients who had the wild type gene. However, the epileptic patients who had the CYP2C9 polymorphism had lower fasting blood sugar levels than those with the wild type gene, although no insulin resistance. In addition, patients with the CYP2C9 polymorphism also had statistically significantly higher ankle brachial indexes than patients with the wild type gene.

The CYP P450 superfamily represents one group of important Phase I drug metabolizing enzymes that oxidize a number of endogenous compounds and xenobiotics, including PHT.[13] The most common polymorphisms that have significant clinical importance are CYP2C9*2 and CYP2C9*3; CYP2C9*1 refers to the wild-type gene. The identified mutant alleles of CYP2C9, CYP2C9*2 and CYP2C9*3 have 70% and 3–5% enzyme activity compared with the wide-type gene, CYP2C9*1.[13] Approximately 40% of the white and 5% of the Asian or black populations are heterozygous for either CYP2C9*2 or CYP2C9*3 and demonstrate a significantly decreased CYP2C9 activity compared with those having CYP2C9*1/*1.[13] Kuanprasert et al. conducted a study on valvular heart disease in Northern Thailand and found that 95% of 242 patients under investigation had CYP2C9*1/*1; CYP2C9*1/*3 was found in the remaining 5%. Neither the mutant CYP2C9*2 allele nor the individual homozygote for CYP2C9*3 were found in this study.[14] Surprisingly, our study found that the CYP2C9 polymorphism had a prevalence of 25%. About 24% (18 out of 75) of epileptic patients were found to be CYP2C9*3 heterozygous carriers (wt/mt) and 1.3% (1 out of 75) were CYP2C9*3 homozygous (mt/mt), which is characterized by the phenotype with poor PHT metabolism. No homozygous or heterozygous CYP2C9*2 were found. Compared with other Asian countries, there was a significantly high frequency of CYP2C9 polymorphism in our study.

Metabolic abnormalities such as increased levels of total cholesterol, total triglyceride and LDL-cholesterol, low levels of HDL cholesterol and high fasting blood sugar, are independent risk factors of atherosclerosis.[15] Previous reports have suggested that vascular risk factors are more prevalent in chronic epilepsy.[16–18] There are a limited number of studies on the effect of PHT on lipid profiles in adult epileptic patients. Nikolaos et al., found that adult epileptic patients with CYP P450 enzyme-inducing AED had increased serum levels of total cholesterol, HDL cholesterol, LDL cholesterol and total triglycerides.[16] In a recent study, we controlled the confounding factors which can effect the lipid profile, and found that PHT patients had an increased mean value of serum total cholesterol, total triglyceride and serum LDL cholesterol as compared to patients with epilepsy taking valproate and those who had not previously taken any AEDs.[4] The mechanism responsible for the change in lipid profile amongst patients taking PHT may be due to the effect of CYP 450 induction.[19] There is a correlation between the serum lipid and hepatic microsomal enzyme activity and CYP450 content in liver biopsy specimens.[20] To date, however, no study has focused on the effect of the CYP2C9 polymorphism on serum lipid profiles. In our study, we found that epileptics who had the CYP2C9*3 polymorphism had statistically significant lower total cholesterol, total triglyceride HDL cholesterol, and LDL cholesterol than epileptic patients who had the wild type of CYP2C9. Interestingly, this result was not associated with the serum PHT levels, suggesting the effect of PHT on the lipid profile does not depend on the PHT itself but is an indirect effect on the CYP P450 enzyme system, which is involved extensively in the synthesis and metabolism of cholesterol.

High serum blood glucose and insulin resistance are the important risk factors of atherosclerosis. The effect of PHT on glucose homeostasis in patients with epilepsy is limited. To date we know of only three studies that have been conducted in this area. Two studies examined glucose tolerance and insulin secretion in epileptic patients who were on long-term PHT therapy, and found no evidence of insulin resistance.[21,22] These studies had numerous limitations, however, notably a lack of BMI comparison between the groups, serum levels of PHT and insulin resistance, and small sample sizes. Our previous study controlled for all of these confounding factors and found that insulin resistance was statistically significantly higher in

epileptic patients taking phenytoin than in epileptic patients taking valproate or epileptic patients who were not taking AEDs.[4] The mechanism of PHT-induced insulin resistance has not been well established. Some evidence has been found showing an association between serum levels of PHT and delays in reaching peak glucose concentrations.[21] In this current study, we analyzed the association of serum levels of PHT and fasting blood sugar, but found no correlation between these levels. We compared the CYP2C9 polymorphism with the wild type gene with fasting blood sugar, and found that the CYP2C9 polymorphism group had statistically significant higher levels than the wild type gene ($p = 0.04$). Also, patients who had a CYP2C9 polymorphism had higher serum insulin levels than patients with the wild type gene ($p = 0.04$), but no statistically significant levels of insulin resistance ($p = 0.12$). The mechanism of association between CYP2C9 polymorphism and serum insulin level needs to be explored further.

Concerning the association between lipid abnormality, insulin resistance and atherosclerosis, we aim to explore the effects of CYP2C9 polymorphism on subclinical atherosclerosis in epileptic patients taking PHT, as measured by the ankle brachial index. The ankle brachial index (ABI) and carotid intima-media thickness are two well-studied markers of atherosclerosis that are useful in risk stratification.[23] Our study found that wild type gene patients taking PHT as monotherapy had a statistically significant lower ABI at the left and right leg than those who had the CYP2C9 polymorphism ($p = 0.03$ and 0.04 respectively). A low ABI ($\leq$0.90) at rest indicates a high risk of peripheral vascular disease and provides significant evaluative and prognostic information on cardiovascular risk. In our study, 34.7% of patients taking PHT had a low ABI at one or both sites. Also, we separated the patients into CYP2C9 polymorphism and wild type gene, and found low ABIs in 10.5% and 42.9%, respectively. Our study finding agrees with studies by Hamed et al.[17] and Tan et al.,[18] which, based on common carotid artery intima media thickness measurements, presented observations to support the significantly association with acceleration of atherosclerosis in patients with epilepsy.

The main limitation of this study was that, due to ethical guidelines, we had no patients who had never received an AED which meant that enrollment of patients with epilepsy who had never received an AED, the most reliable control, was simply not an option.

There are two notable strengths of this study. It is the first study to focus on the effect of the CYP 2C9 polymorphism on metabolic abnormalities and subclinical atherosclerosis in epileptic patients taking PHT as monotherapy, and we demonstrated that epileptic patients who had the CYP2C9 polymorphism had lower total triglyceride, total cholesterol, LDL cholesterol, and HDL cholesterol levels than epileptic patients who had the normal CYP2C9 genotype. In addition, patients with the wild type also had statistically significantly lower ABI than patients with the CYP2C9 polymorphism. This study also excluded patients with potential confounding factors that can affect atherosclerosis, thus our finding that among young epileptic patients taking PHT, those with the CYP2C9 polymorphism are at lower risk for atherosclerosis than those with the wild type. These findings may have clinical relevance to atherosclerosis in epileptic patients who are taking PHT in populations with a high prevalence of CYP 2C9 polymorphism such as among Thais who live in Southern Thailand compared with populations in other Asian countries or other regions of Thailand.

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