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CYP2C9, CYP2C19 and ABCB1 Genotype and Hospitalization for Phenytoin Toxicity

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INTRODUCTION

Phenytoin is a narrow therapeutic index drug with nonlinear pharmacokinetics. It is metabolized to 5-(4-hydroxyphenyl)-5-phenylhydantoin principally by cytochrome P450 (CYP) 2C9 and less so by CYP2C19.[1] There is conflicting evidence about the potential role of P-glycoprotein (coded by the adenosine triphosphate-binding cassette sub-family B member 1; ABCB1) in transporting phenytoin out of the central nervous system (CNS).[2–7] Although a number of studies have examined associations between single nucleotide polymorphisms (SNPs) of these genes and serum phenytoin concentrations,[8–11] we are unaware of any studies that have looked for an association between genotype and acute CNS toxicity.

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Therefore, we sought to examine the association between common genetic variants in the exons of CYP2C9, CYP2C19 and ABCB1 genes and the risk of acute CNS toxicity leading to hospitalization in persons receiving phenytoin.

METHODS

Overview

We conducted a case-control study nested within two different cohorts of users of phenytoin. The first cohort was a prospectively-enrolled cohort of elderly (≥ 65 years) new and continuing phenytoin users who were beneficiaries of either the Pennsylvania Pharmaceutical Assistance Contract for the Elderly (PACE) program or the PACE Needs Enhancement Tier (PACENET) program in the Unites States (US). Henceforth, both PACE and PACENET will be referred to as PACE. The assembly of this cohort has been described elsewhere.[12] The second cohort consisted of elderly and non-elderly phenytoin users identified within Geisinger Health System (GHS), a rural health maintenance organization located in northeast and central Pennsylvania. New and continuing phenytoin users within GHS were prospectively identified during 2001–2006 via prescription orders in GHS' ambulatory electronic medical record system. The PACE and GHS cohorts served as the base from which cases and controls were selected. This research was approved by the University of Pennsylvania's Committee on Studies Involving Human Beings and by GHS' Office for Human Research Protection. Each study participant provided written informed consent and Health Insurance Portability and Accountability Act authorization. Except for comments received during the peer review process for obtaining funding, the funding sources of this study had no role in its design, conduct, or interpretation.

Identification and Enrollment of Cases and Controls

Potential cases were identified by the presence of a discharge International Classification of Diseases, 9th Revision, (ICD9) diagnostic code indicating a hospitalization for phenytoin toxicity (966.1 or E936.1) in either a principal or non-principal position. These data were obtained from the Pennsylvania Healthcare Cost Containment Council (for PACE subjects) and the EpicCare in-hospital electronic medical record system (for GHS subjects). Two reviewers (S.H. and C.E.L.) independently reviewed hospital discharge summaries of potential cases to ascertain outcome occurrence. The outcome definition was met when the record indicated presence of abnormal CNS findings (e.g., nystagmus, ataxia, confusion, decreased consciousness, lethargy, dullness, drowsiness, hallucinations, hyperactivity, mental status change) at the time of original clinical presentation leading to the hospital admission; such findings were attributed by a treating clinician as possibly or definitely due to phenytoin; and the findings developed as a result of ambulatory rather than in-hospital phenytoin administration. Initial disagreements were settled by consensus. Controls were members of the underlying cohort (phenytoin recipients in either the PACE or GHS) who had no identified hospitalizations for phenytoin toxicity during the study period.

Cases and controls identified in the PACE population were recruited via telephone and mail and enrolled from March through November 2004. Subjects identified in GHS were

recruited similarly and enrolled from June through December 2006. Participants were remunerated with $10 in the form of a money order or gift card.

Identification of Exposure

Subjects were mailed buccal swabs for DNA collection, used the swabs per written directions to collect biosamples, and returned them via mail to investigators. Subjects' genotypes for CYP2C9, CYP2C19, and ABCB1 were determined as described below. As individuals carry two copies of each gene, subjects were categorized into one of six groups for each CYP enzyme (*1/*1, *1/*2, *1/*3, *2/*2, *2/*3, *3/*3) and one of three groups for ABCB1 (C/C, C/T, C/T), with *1 and C representing the non-variant allele. These SNPs were chosen for study because of their potential role in the pathogenesis of phenytoin toxicity and their anticipated allele frequencies. For example, CYP2C9*2 (under study) is expected in 15% of whites,[13] while CYP2C9*6 (not under study) is not an expected variant (0%).[14]

Laboratory Procedures

DNA was extracted via standard laboratory protocols. Genotyping was performed for CYP2C9*2 (rs1799853), CYP2C9*3 (rs1057910), CYP2C19*2 (rs4244285), CYP2C19*3 (rs4986893), and ABCB1 C3435T (rs1045642) on a 7500 Real-Time PCR System (Applied Biosystems: Foster City, California). DNA was genotyped according to the manufacturer suggested protocol for Drug Metabolism Genotyping Assays. Briefly, the reaction components for each genotyping reaction were as follows: 10 nanograms of DNA, 5 microliters (μL) of TaqMan Genotyping Master Mix, 0.5 μL of assay mix, and water to a total volume of 10 μL. The thermocycler conditions were as follows: 50°C for 2 minutes, 95°C for 10 minutes, and 50 cycles of 92°C for 15 seconds and 60°C for 90 seconds. Randomized sample duplications between plates were set to check the accuracy of the genotyping. The results were then analyzed by Applied Biosystems Sequence Detection Software after post-reading and tested for deviation from Hardy-Weinberg equilibrium with the Helix Tree software package (Golden Helix: Bozeman, Montana) using a p-value <0.01. Deviation from Hardy-Weinberg equilibrium was not identified for any genotype examined.

Statistical Analysis

We first compared cases and controls with respect to recruitment source, demographic factors, and genotype. We then calculated unadjusted odds ratios (ORs) with 95% confidence intervals (CIs) for the association of each genotype with phenytoin toxicity, using the homozygous non-variant genotype as the reference category. We then used logistic regression to adjust for potential confounding. Statistical analyses were conducted using Stata version 9 (StataCorp LP: College Station, Texas). Based on pilot data, we anticipated enrolling 80 cases and 320 controls, which would have yielded 80% statistical power to detect an OR of 2.4 for having at least one copy of CYP2C9*2 (expected prevalence: 15%[7]) and an OR of 2.2 for having at least one copy of ABCB1 C3435T (expected prevalence: 62%[15]), with a type-1 error rate of 5%.[16]

RESULTS

Within PACE, we identified 38 validated cases, yet enrolled only five. Reasons for non-enrollment included refusal to consent (N = 16), unable to contact subject (N = 10), and subject deceased at the time of contact (N = 7). The five validated cases were matched to 274 persons serving as controls, for a total of 279 PACE enrollees. Within GHS, we identified 27 validated cases, yet enrolled only nine. Reasons for non-enrollment included refusal to consent (N = 8), unable to contact subject (N = 6), and subject deceased at time of contact (N = 4). The nine validated cases were matched to 16 persons serving as controls, for a total of 25 GHS enrollees. Thus, we enrolled a total of 14 cases and 290 controls. Characteristics of cases and controls are listed in Table 1.

Table 1 also presents unadjusted and adjusted ORs for each observed genotype. The adjusted OR for the CYP2C9 *1/*3 genotype was 8.91 (95% CI, 0.79 to 100.0). The adjusted OR for the CYP2C9 *2/*2 genotype was 9.48 (95% CI, 0.79 to 114.5). The adjusted OR for the CYP2C19 *1/*3 genotype was 4.21 (95% CI, 0.58 to 30.8). All of the other ORs were close to one. A sensitivity analysis excluding non-white subjects yielded similar point estimates (data not shown).

DISCUSSION

Our ability to make inferences about the role of CYP2C9, CYP2C19, and ABCB1 was hindered by the limited number of cases identified and enrolled. Limited identification may have been due in part to a secular trend in the number of hospitalizations for phenytoin toxicity. For example, using data from the Nationwide Inpatient Sample database of the Healthcare Cost and Utilization Project (http://www.hcup-us.ahrq.gov/nisoverview.jsp), which includes about 90% of community hospital discharges in the US, we found that the number of hospitalizations with a discharge diagnosis of phenytoin toxicity (ICD9 code 966.1) declined from 8,177 in 1994 to 3,645 in 2006.[17] The sensitivity of the ICD9 codes we used to identify phenytoin toxicity was previously reported to be 87%.[18] Limited enrollment may have been due in part to the advanced age and cognitive status of patients taking phenytoin, making obtaining consent more difficult.

The adjusted odds ratio of 8.91 for CYP2C9 *1/*3 and of 9.48 for CYP2C9 *2/*2 should both be interpreted very cautiously given that neither achieved statistical significance, and that they are each based on only a single case with that genotype. Further, given the wide CIs associated with the ORs for other genotypes, this study certainly neither demonstrates nor excludes a potential role of the genes studied in the etiology of acute CNS toxicity of phenytoin.

Future studies of this question should ideally include a larger number of events, and therefore may need to be conducted across multiple sites throughout the US and abroad, and identify strategies to overcome barriers to subject recruitment.

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Financial Disclosures:

Dr. Hennessy has served as a consultant to a law firm representing Pfizer, and to Sanofi-Synthelabo and Teva Pharmaceuticals, (manufacturers of phenytoin) in matters unrelated to phenytoin. Dr. Strom has consulted for the following manufacturers of phenytoin, in matters unrelated to phenytoin: Abbott Pharmaceuticals, Pfizer, Sanofi-Synthelabo, and Teva Pharmaceuticals.

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Table 1

Characteristics of cases for hospitalization for neurologic phenytoin toxicity and controls and results of multivariable logistic regression models

	n (%)			
	Cases N = 14	**Controls N = 290**		
Recruitment source				
PACE[†]	5 (35.7)	274 (94.5)		
Geisinger Health System	9 (64.3)	16 (5.5)		
Sex				
Male	4 (28.6)	79 (27.2)		
Female	10 (71.4)	211 (72.8)		
Race				
White	14 (100)	274 (94.5)		
African American	0 (0.0)	13 (4.5)		
Other	0 (0.0)	2 (0.7)		
Unknown	0 (0.0)	1 (0.3)		
Median age, in years (interquartile range)	68.4 (33.9)	76.3 (10.2)		
Mean phenytoin concentration on admission[‡], mg/L (standard deviation)	31.2 (12.9)	-		
			Crude OR (95% CI)	Adjusted[§] OR (95% CI)
Genotype				
CYP2C9				
*1/*1	8 (57.1)	194 (66.9)	1.00 (reference)	1.00 (reference)
*1/*2	4 (28.6)	89 (30.7)	1.09 (0.32–3.71)	0.96 (0.25–3.66)
*1/*3	1 (7.15)	4 (1.4)	6.06 (0.61–60.6)	8.91 (0.79–100)
*2/*2	1 (7.15)	3 (1.0)	8.08 (0.75–86.6)	9.48 (0.79–115)
*2/*3	0 (0.0)	0 (0.0)	-	-
*3/*3	0 (0.0)	0 (0.0)	-	-
CYP2C19				
*1/*1	10 (71.4)	213 (73.5)	1.00 (reference)	1.00 (reference)
*1/*2	2 (14.3)	70 (24.1)	0.61 (0.13–2.84)	0.50 (0.09–2.73)
*1/*3	2 (14.3)	7 (2.4)	6.09 (1.12–33.1)	4.21 (0.58–30.8)
*2/*2	0 (0.0)	0 (0.0)	-	-
*2/*3	0 (0.0)	0 (0.0)	-	-
*3/*3	0 (0.0)	0 (0.0)	-	-
ABCB1				
C/C	3 (21.4)	72 (24.8)	1.00 (reference)	1.00 (reference)
C/T	6 (42.9)	123 (42.4)	1.17 (0.28–4.82)	0.84 (0.18–3.81)
T/T	5 (35.7)	95 (32.8)	1.26 (0.29–5.46)	1.32 (0.28–6.18)

[†]Pennsylvania Pharmaceutical Assistance Contract for the Elderly prescription drug program (including members of the Needs Enhancement Tier)

‡Usual therapeutic range = 10 to 20 mg/L

Adjusted for age, sex, and number of variant alleles of other metabolic enzymes

OR = odds ratio; CI = confidence interval