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SHORT COMMUNICATION

Superoxide dismutase 2 Val16Ala polymorphism is a risk factor for the valproic acid-related elevation of serum aminotransferases

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γ-Glutamyltransferase

Summary The association between the *superoxide dismutase 2* (*SOD2*) Val16Ala polymorphism and the serum aminotransferase levels was retrospectively investigated in 207 valproic acid-treated patients with epilepsy. The Val/Val genotype tended to show elevated alanine aminotransferase levels (odds ratio = 3.5; $P = 0.056$). In addition, an elevated γ-glutamyltransferase level was associated with the Val/Val genotype (odds ratio = 3.1; $P = 0.022$). The *SOD2* Val/Val genotype may therefore contribute to a valproic acid-induced elevation in the serum aminotransferase levels.

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Introduction

Valporoic acid (VPA) is one of the most widely prescribed antiepileptic drugs worldwide (Chateauvieux et al., 2010). VPA has numerous side effects such as weight gain and hepatotoxicity (Silva et al., 2008; Chateauvieux et al., 2010; Begriche et al., 2011). Recently, non-alcoholic fatty liver disease (NAFLD) has emerged as a common chronic liver condition in VPA-treated patients (Luef et al., 2004, 2009; Verrotti et al., 2011).

Mitochondrial dysfunction has been implicated in the pathogenesis of VPA-induced hepatotoxicity (Silva et al., 2008; Begriche et al., 2011). Superoxide dismutase 2 (SOD2, E.C. 1.15.1.) plays a critical role in the detoxification of mitochondrial reactive oxygen species (Lee et al., 2010; Begriche et al., 2011). Extensive research had been conducted using *Sod2* mutant mouse models for defining various oxidative stress-induced disorders including liver diseases (Lee et al., 2010). The T to C nucleotide polymorphism (rs4880, Val16Ala) has been identified in exon 2 of the human *SOD2* gene, and the Ala variant is more efficiently imported into the mitochondria than the Val variant, thus resulting in increased mitochondrial SOD2 homotetramer activity derived from the Ala precursor variant (Sutton et al., 2003). The *SOD2* Val/Val genotype has been proposed to be a risk factor for susceptibility to non-alcoholic steatohepatitis (Namikawa et al., 2004).

This study was designed to determine whether the *SOD2* Val16Ala polymorphism is associated with liver dysfunction in VPA-treated patients with epilepsy.

Subjects and methods

The study included 207 Japanese patients with epilepsy (80 females and 127 males), who were found to have no history of either viral or alcoholic liver disease and had received VPA therapy for over one month at Kumamoto Saishunso National Hospital. The demographic and clinical information between May 1991 and July 2008 were obtained retrospectively from the medical records. The institutional ethics committee approved this study. All of the patients provided their written informed consent to participate in the study.

The maximum value of alanine aminotransferase (ALT) for each patient during the study period and the values of aspartate aminotransferase (AST) and γ-glutamyltransferase (γGT) at that time were used for the statistical analyses. All of the levels of aminotransferases were measured by the standard methods recommended by the Japan Society of Clinical Chemistry in daily practice at Kumamoto Saishunso National Hospital. Elevated aminotransferase was defined as an increase over the upper limit of the normal range (ULN), which is stratified by age and sex (Wallach, 1996).

Genomic DNA was extracted from whole blood using a DNA purification kit (Flexi Gene DNA kit, QIAGEN, Hilden, Germany). The genotypes of *SOD2* were determined using the polymerase chain reaction restriction fragment length polymorphisms methods as previously reported (Namikawa et al., 2004).

The genotype distribution of *SOD2* polymorphism was tested for Hardy-Weinberg equilibrium using the χ^2 test. The data are presented as the medians (range) or the number of the patients' characteristics. All continuous variables were analyzed using the Mann—Whitney *U* test. The categorical variables were compared by Fisher's exact test. The strength of the association between the *SOD2* genotypes and the risk of the elevated aminotransferases over ULN were measured as the odds ratios (ORs) with 95% confidence intervals (CI) using a logistic regression analysis. The ORs were adjusted by potentially confounding factors, such as age, sex, body mass index, VPA dose/body weight, duration of VPA treatment, and the co-administration of enzyme-inducing antiepileptic drugs (i.e. carbamazepine, phenytoin, phenobarbital and primidone) or non-enzyme-inducing antiepileptic drugs. A P value of <0.05 was considered to be statistically significant. All statistical analyses were performed using the SPSS software package (version 17.0, SPSS Inc., Chicago, IL, USA).

Results

The frequencies of the *SOD2* Val/Val, Val/Ala and Ala/Ala genotypes were 78.3%, 19.8% and 1.9%, respectively. The distribution of the three genotypes was in Hardy-Weinberg equilibrium. The number of patients with the Ala/Ala genotype (4 patients) was too small to assess the effect of the genotype on the serum aminotransferases; therefore, the Ala/Ala and the Val/Ala genotypes were combined in the subsequent statistical analyses. Table 1 shows the patients' characteristics at the time when the ALT reached the maximum value for each patient during the VPA treatment. None of the patients' characteristics were significantly different between the *SOD2* Val/Val and the other *SOD2* genotypes, except for the serum levels of AST (Table 1).

Table 2 shows the association between the *SOD2* genotype and the risk of the elevated aminotransferases over ULN. The frequency of cases with elevated ALT tended to be higher in the *SOD2* Val/Val genotype than that in the other genotypes. The elevated γGT was significantly associated with the *SOD2* Val/Val genotype.

Additional analyses excluding 43 patients (20.8%) treated with a VPA daily dosage over the recommended limit (i.e. 30 mg/kg for children or 1200 mg for adults) revealed an association between the elevated ALT and γGT, and *SOD2* Val/Val genotype (adjusted OR = 10.5 and 4.0, 95% CI 1.2—89.7 and 1.4—11.5, respectively). The risk for the elevated aminotransferases did not differ between the *SOD2* genotypes in the excluded patients with a high dose of VPA (data not shown). No association was observed between the elevated AST and *SOD2* genotypes in any of the analyses.

Discussion

This is the first report to show that the functional polymorphism in *SOD2* gene was associated with the VPA-induced elevation of serum aminotransferases. The risk for the elevation of ALT and/or γGT was three-fold higher in the *SOD2* Val/Val genotype than the others.

The potential hepatotoxicity of VPA is a major concern (Silva et al., 2008; Chateauvieux et al., 2010; Begriche et al., 2011). Long-term treatment with VPA is associated with high prevalence of NAFLD in adolescents (36.0%) (Verrotti et al., 2011) and adults (60.9%) (Luef et al., 2004, 2009) with epilepsy. Meanwhile, a recent study demonstrated the serum ALT or γGT levels to be slightly but significantly higher in obese children with NAFLD than in those without NAFLD, and the degree of liver fatty infiltration has been shown to positively correlate with these aminotransferases (Radetti et al., 2006). A mild increase