Phenoconversion of *CYP2C9* in epilepsy limits the predictive value of *CYP2C9* genotype in optimizing valproate therapy

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Abstract

Aims: Since prominent role in valproate metabolism is assigned to CYP2C9 in pediatric patients, the association between children's CYP2C9-status and serum valproate concentrations or dose-requirements was evaluated.

Methods: The contribution of *CYP2C9* genotype and CYP2C9 expression in children (N=50, Caucasian) with epilepsy to valproate pharmacokinetics was analyzed.

Results: Valproate concentrations were significantly lower in normal expressers with *CYP2C9*1/*1* than in low expressers or in patients carrying polymorphic *CYP2C9* alleles. Consistently, the dose-requirement was substantially higher in normal expressers carrying *CYP2C9*1/*1* (33.3 mg/kg *vs* 13.8-17.8 mg/kg, P<0.0001). Low CYP2C9 expression significantly increased the ratio of poor metabolizers predictable from *CYP2C9* genotype (by 46%).

Conlusion: Due to the substantial down-regulation of CYP2C9 expression in epilepsy, inferring patients' valproate metabolizing phenotype merely from *CYP2C9* genotype results in false prediction.

Keywords: personalized medication, epilepsy, pediatric patients, valproate therapy, cytochrome P450, CYPtest, *CYP2C9* genotype, CYP2C9 expression

Abbreviations: CYP cytochrome P450; VPA valproate;

Introduction

One percent of Hungarian pediatric population has been reported to suffer from epilepsy [1], but most of them are treated successfully with anticonvulsants. One of the first choices of antiepileptic therapy is valproic acid (VPA), which is generally well-tolerated, and rarely induces serious side effects. Rare complications may occur in patients treated chronically with VPA, including hepatotoxicity, hematologic disorders, hyperammonemic encephalopathy or neurological toxicity [2,3]. The risk of serious adverse effects is increased in children, especially in those younger than 2 years of age. The mechanism of VPA-induced toxicity is not clearly understood, but both the parent compound and some of its unsaturated metabolites have been associated with mitochondrial dysfunction and cytotoxicity [4].

VPA, the branched short-chain fatty acid, is extensively metabolized in the liver, resulting in conjugated, unsaturated and hydroxylated metabolites [5,6]. In adults, the majority of VPA dose is eliminated as glucuronide conjugate in the urine. Mitochondrial β-oxidation is the second major route of biotransformation, forming 2-ene-VPA, 2,4-diene-VPA and 3-keto-VPA. The cytochrome P450 (CYP) mediated branch of VPA metabolism is the formation of 4-ene-VPA and hydroxylated metabolites (3-, 4-, and 5-hydroxy-VPA metabolites) [7,8]. Kiang *et al.* have demonstrated that CYP2C9 is the major enzyme in CYP-mediated metabolism of VPA, accounting for about 10-15% of the administered dose, whereas CYP2A6 and CYP2B6 play a minor role in VPA metabolism [9]. Although CYP-mediated pathways contribute to a minor part of VPA metabolism in adults (less than 20% of the administered dose), the CYP-catalyzed oxidation may become the principal route of the metabolism in those special cases when glucuronidation or mitochondrial β-oxidation pathways are compromised or poorly developed, for example, in children. Shifting the metabolic pathways may account for the age-related differences in the incidence of VPA-induced adverse effects. i) Hepatic glucuronidation is known to be developmentally regulated.

UDP-glucuronyl transferases involved in VPA glucuronidation [10], are expressed under the adult levels until sometime after 10-15 years of age [11,12]. *In vitro* glucuronide conjugation of VPA has been demonstrated to be catalyzed by UGT1A6, UGT1A9 and UGT2B7 [10]; and Guo et al. have confirmed the role of UGT1A6 *in vivo*; however, UGT2B7 seems to catalyze VPA glucuronidation less efficiently [13]. ii) VPA and some of its metabolites are considered to be the inhibitors of mitochondrial β-oxidation [14]. iii) CYP-dependent metabolism in children exceeds adult activities, and decreases to adult levels by puberty [15]. As a consequence, larger amount of VPA dose is liable to CYP2C9-dependent metabolism in pediatric patients than in adults. Furthermore, the genetic and non-genetic factors, influencing CYP2C9 activity, can increase the predisposition to VPA-induced serious adverse reactions; thus, recognition of risk factors can contribute to the avoidance of adverse events.

There have been several clinical studies, investigating relationship between VPA pharmacokinetics and patients' *CYP* genotypes, although clear evidence for the association between VPA serum concentrations and *CYP2C9* genotype has been rarely provided [13,16]. Statistically significant, but relatively small differences in plasma concentrations of VPA have been observed in patients with *CYP2C9*3* allele comparing to those with two wild type alleles [16]. Although polymorphic *CYP* alleles result in non-functional CYP enzymes and permanent poor metabolism, the individuals with functional wild type alleles may become transient poor metabolizers as an effect of internal (e.g. diseases, hormonal status) or environmental factors (e.g. nutrition, medication). This means that *CYP* genotype determines the potential for the expression of functional or non-functional CYP enzyme. For example, a patient with *CYP2C9*2/*2* or *CYP2C9*3/*3* basically displays poor metabolism of CYP2C9 substrates, whereas a subject carrying *CYP2C9*1/*1* possesses the potential for having functional CYP2C9 enzyme. However, non-genetic factors, such as co-medications or co-morbidities give rise to altered phenotypes. Thus, *CYP2C9*1/*1* genotype, predicted to be

translated to an extensive metabolizer phenotype, may be switched into poor metabolism due to phenoconversion, which eventually influences the patient's response to VPA [17]. Furthermore, the genotype-phenotype mismatch results in more poor metabolizers than it would be predicted from *CYP2C9* genotype.

A patient's CYP-status can be estimated by the evaluation of *CYP* genotypes and current CYP expression. We have previously described a complex diagnostic system (CYPtest^{TM}) that can determine drug metabolizing capacity by combining *CYP* genotypes and current CYP expression in leukocytes [18]. CYP2C9 mRNA levels in leukocytes of those subjects who do not carry loss-of-function mutations in *CYP2C9* gene was proven to reflect the hepatic tolbutamide hydroxylation activity selective for CYP2C9 [18]. A preliminary *CYP2C9* genotyping for *CYP2C9*2* and *CYP2C9*3* can identify the genetically determined poor metabolism of CYP2C9 enzyme, and then CYP2C9 expression in leukocytes of patients with wild type alleles (*CYP2C9*1/*1*) can estimate a reduced or even increased CYP2C9 activity resulted by non-genetic variations. A patient carrying *CYP2C9*1/*1* genotype can be assumed to be an extensive metabolizer and able to biotransform VPA more rapidly than others carrying polymorphic *CYP2C9*2* or *CYP2C9*3* alleles. However, non-genetic factors can modify the expression of the functional wild type alleles resulting in transient poor metabolism similarly to those with non-functional polymorphic *CYP2C9* alleles. In the present study, we investigated CYP2C9-status of pediatric patients younger than 15 years of age and its influence on the steady-state serum concentrations of VPA as well as on patients' dose-requirements. We attempted to provide evidence for that *CYP2C9* genotype is not the only determinant factor in CYP2C9 metabolizer status of a patient, but the expression rate of the wild type gene can highly influence a patient's CYP2C9 metabolizing capacity and his/her response to a drug.

Patients & methods

- **Patients and sampling procedures**

Pediatric patients (N=50) suffering from epilepsy diagnosed with partial or generalized seizures were enrolled in the study carried out at Heim Pál Children's Hospital and at the 2nd Department of Pediatrics, Semmelweis University (Budapest, Hungary). We recruited novel epileptic patients, younger than 15 years of age, who were CYP2C9 tested at the beginning of antiepileptic therapy. The patients on non-VPA therapy or on multi-drug therapy were excluded from the study. The patients were also excluded if their VPA therapy was interrupted. The parents or representatives of each pediatric patient gave their informed consent to participate in this study.

The patients' demographic data, as well as the details of anticonvulsant therapy were recorded. The patients (boys/girls: 20/30) were at the average age of 6.75 years (range: 0.5 – 15 years), and all of them belonged to the Caucasian white population. Blood samples for CYP2C9 testing were taken before the beginning of anticonvulsant therapy. The patients were not given any other medication, but VPA as mono-therapy, and the target dose was adjusted to the patients' body weight according to the clinical protocol [19]. The therapy was initiated at low dosages (10-15 mg/kg), and the target doses were subsequently titrated until optimal clinical response was achieved, generally within 5-10 days. Blood samples for drug assays were taken two and four weeks after the beginning of VPA treatment. The sampling at the second week was applied for checking VPA serum concentration, and the dose was modified if the exposure exceeded the target range of VPA concentration. The serum levels measured at the fourth week were considered to be the stable steady-state concentrations, whereas the doses applied for the stable VPA concentrations were considered to be the maintenance doses.

- **CYP2C9 testing**

Patients' CYP2C9-status was determined by *CYP2C9* genotyping and by assaying

CYP2C9 expression in leukocytes before the beginning of VPA administration. Genomic DNA and leukocytes were isolated from the samples of peripheral blood according to the methods described by Temesvári et al. [18]. *CYP2C9* genotyping was carried out by hydrolysis single nucleotide polymorphism analysis for *CYP2C9*2* and *CYP2C9*3* using TaqMan Probes (BioSearch Technologies, Novato CA). For CYP2C9 expression, total RNA was isolated from leukocytes, RNA was reverse-transcribed into single-stranded cDNA, and then real-time PCR with human cDNA was performed using UPL probe for CYP2C9 (Roche Diagnostics, Mannheim, Germany). The quantity of CYP2C9 mRNA relative to that of the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase was determined. Three categories of CYP2C9 expression were applied to describe low, normal and high expressers. The cutoff values for the CYP2C9 mRNA levels in leukocytes were previously established on the basis of the cutoff values for the hepatic CYP2C9 activity (tolbutamide hydroxylation), allowing a distinction between low, normal (medium) and high expressers ($5*10^{-6}$ and $2.5*10^{-5}$, respectively) [18].

- **Serum VPA assay**

The blood samples were taken before the patients were administered the morning dose. The steady-state serum concentration of VPA was determined by the fluorescence polarization immunoassay method (AxSYM Valproic Acid Assay, Abbott Laboratoties, IL). The VPA concentrations ranged between 40 and 100 μg/ml were considered to be the therapeutic levels [19].

- **Statistical analysis**

The serum concentration values of VPA were normalized by the dose and the body weight, and expressed as (μg/ml) × (mg dose/kg body weight)$^{-1}$. The data of normalized VPA concentrations and dose-requirements for the optimal therapeutic level in the groups with various CYP2C9-statuses were expressed as the median (and range). It should be noted that

median values did not differ much (generally by 1-2% and always under 5%) from the mean values. Between-group differences were calculated by the use of Kruskal-Wallis test followed by Dunn's multiple comparisons test. A P value of <0.05 was considered statistically significant.

Results

CYP2C9-status of pediatric patients

Of 50 pediatric patients aged between 0.5 and 15 years, all expressed at least one functional *CYP2C9* allele, and 70% of patients carried *CYP2C9*1/*1* genotype (Table 1). The patients with two loss-of-function alleles were not enrolled in the study, since they were on non-VPA therapy. Fifteen patients (30%) carried one of the polymorphic variant alleles (*CYP2C9*2* or *CYP2C9*3*). The frequencies of *CYP2C9*2* and *CYP2C9*3* alleles in patients (9% and 6%, respectively) were similar to those in Caucasian (white) populations (11% and 7%, respectively) [20,21]. CYP2C9 expression assays revealed that almost half of the patients (46%, N=23) were normal CYP2C9 expressers, and substantial portion of the patients (54%, N=27) were low expressers (Table 1). None of the children displayed high CYP2C9 expression. On the basis of CYP2C9-status (*CYP2C9* genotypes and CYP2C9 expression), the patients were grouped into two main categories - homozygous wild (*CYP2C9*1/*1*) and heterozygous *CYP2C9*1/mut* genotypes (*CYP2C9*1/*2* or *CYP2C9*1/*3*), - and subdivided into two subgroups: normal (medium) and low CYP2C9 expressers (Table 1). Although patients with two wild type alleles are generally considered to be extensive metabolizers, merely 12 children of 35 patients with *CYP2C9*1/*1* genotype were found to be normal CYP2C9 expressers, whereas the other 23 patients were low expressers, predicting poor CYP2C9 metabolism. Furthermore, the group of patients with heterozygous *CYP2C9*1/mut* genotypes comprised both low and normal CYP2C9 expressers (4 and 11 patients, respectively). It is not surprising, since the mutant alleles are transcribed into CYP2C9 mRNA; however, their expression rates are modified by non-genetic factors, such as nutrition, food additives, or hormonal status, similarly to the wild type allele. Co-medication as a non-genetic factor can be excluded, since the children on multi-drug therapy were not enrolled in the present study.

Patients' VPA exposure and dose-requirement

The statistical analysis displayed significant association between the patients' CYP2C9-status and the steady-state serum levels of VPA normalized by the dose and the body weight. The normalized serum VPA concentrations were significantly lower in the normal expresser patients with *CYP2C9*1/*1* genotype (2.12 (μg/ml)×(mg dose/kg bw)$^{-1}$) than in low expressers (5.13 (μg/ml)×(mg dose/kg bw)$^{-1}$) or in patients carrying any polymorphic *CYP2C9* alleles (*CYP2C9*2* or *CYP2C9*3*) (4.33 (μg/ml)×(mg dose/kg bw)$^{-1}$ for normal CYP2C9 expressers and 5.54 (μg/ml)×(mg dose/kg bw)$^{-1}$ for low expressers) (Figure 1). The low expressers and the patients with polymorphic *CYP2C9* alleles showed about 2- to 3-fold higher normalized serum VPA levels as compared to normal expresser patients carrying *CYP2C9*1/*1* genotype. The difference in normalized serum concentrations was not statistically significant between the patients with heterozygous genotypes (*CYP2C9*1/*2* or *CYP2C9*1/*3*) and those low expressers with two functional alleles (*CYP2C9*1/*1*). Moreover, no significant difference in normalized serum levels was observed between normal and low expressers with heterozygous *CYP2C9* genotypes.

According to the clinical practice, VPA serum concentrations ranged between 40 and 100 μg/ml are considered to be therapeutically optimal in the management of epilepsy [19]. The low expresser patients or subjects with heterozygous genotypes required significantly lower dose of VPA for the optimal serum level than normal expressers carrying *CYP2C9*1/*1* genotype (Figure 2). The dose-requirement of VPA for the target serum level was similar for the low expressers and for the patients carrying polymorphic *CYP2C9* alleles (17.8 mg/kg for low expressers carrying *CYP2C9*1/*1*; 16.7 mg/kg for normal expressers with heterozygous genotype; 13.8 mg/kg for low expressers with heterozygous genotype). The conventional clinical practice is to target the VPA dose of 30 to 40 mg/kg in children. The conventional dosing approach was appropriate for normal CYP2C9 expresser patients

with *CYP2C9*1/*1* genotype, comprising 24% of the children in the study. The *CYP2C9* genotype-controlled VPA dosing would have targeted reduced VPA dose for 30% of the patients, for those carrying heterozygous *CYP2C9*1/mut* genotypes. However, low expressers with *CYP2C9*1/*1* genotype also required reduced VPA dose for the optimal serum concentration. CYP2C9 phenoconversion substantially increased the number of children (to 76%) on reduced VPA dose.

Multiple comparison analysis showed that CYP2C9-status (*CYP2C9* genotype and CYP2C9 expression) influenced the serum concentrations of VPA as well as the dose-requirements for the optimal serum concentration in pediatric patients. However, low CYP2C9 expression in patients with homozygous wild genotype seemed to display similar effects on VPA exposure and dose-requirement to those carrying polymorphic *CYP2C9* alleles (*CYP2C9*2* or *CYP2C9*3*). Consistently, the serum VPA concentration and dose-requirement of the children carrying two wild type *CYP2C9* alleles (*CYP2C9*1/*1*) were found to be influenced by the CYP2C9 expression, whereas loss-of-function mutations in *CYP2C9* gene resulted in poor metabolism of VPA independently on the degree of CYP2C9 expression.

Discussion

Drug metabolizing capacity highly influences the patient's response to a drug and the risk of side effects. Genetic and non-genetic factors in drug metabolism give rise to substantial interindividual variability in clinical response of drugs, assigning the patient populations into three groups: poor, intermediate and extensive metabolizers [22]. By recognizing individual differences, personalized medication can help to avoid the therapeutic failure or potential adverse reactions [23]. Pharmacogenetic assays can determine poor drug metabolism by genotyping, identifying non-functional drug metabolizing enzymes [22], but

do not provide reliable information about the drug metabolizing capacity of patients who do not have loss-of-function mutations. Non-genetic factors, such as age, diseases, nutrition, or co-medication, can transiently modulate patient's drug metabolizing capacity. Developmental regulation of drug metabolizing enzymes is known to contribute to age-related differences in drug efficacy or toxicity between children and adults [24]. CYP-dependent metabolism is generally low at birth (about 50-70% of adult levels); however, CYP enzyme activities exceed the adult values by the age of 2 years and decrease by puberty [15]. In contrast, the drug-conjugating activities of several UDP-glucuronyl transferases are low or negligible around birth, slightly increasing, but not reaching the adult levels until puberty [11,12]. Concerning VPA, the major metabolic pathway in adults, glucuronidation can shift toward CYP-dependent oxidation in pediatric patients because of reduced glucuronidation ability. On the other hand, chronic administration of VPA leads to the inhibition of β-oxidation pathway of VPA metabolism, assigning a prominent role in the metabolism to CYP enzymes [14,25].

CYP2C9, the main catalyst of CYP-dependent metabolism of VPA, is highly polymorphic with *CYP2C9*2* and *CYP2C9*3* being identified as the most frequent variants in Caucasian population [20,21]. These loss-of-function mutations have been reported to be less active in *in vitro* metabolism of VPA than the wild type allele [26]. The influence of *CYP2C9*3* allele on VPA plasma levels was displayed in Chinese patients [16]; however, the moderate increase in normalized VPA concentrations in the patients carrying *CYP2C9*1/*3* may be attributed to the facts that the authors took neither the CYP2C9 expression nor the age-related differences in VPA metabolism into account. Predicting drug metabolizing phenotype from genotype seems to be highly complex even in the case of non-inducible enzymes, such as CYP2D6 [27]; thus, inferring a patient's VPA metabolizing phenotype merely from *CYP2C9* genotype can easily lead to false interpretations. We have previously reported a more than 60-fold difference in CYP2C9 mRNA levels in human liver tissues

which means that transient poor metabolizers (low CYP2C9 expressers) exist in the group of patients carrying *CYP2C9*1/*1* genotype [18]. Thus, not only genetic, but non-genetic variations of CYP2C9 are of particular importance in the evaluation of patients' CYP2C9-status. The pediatric patients in the present study was divided into two *CYP2C9* genotype groups (*CYP2C9*1/*1* and *CYP2C9*1/mut*), although both groups comprised low and normal CYP2C9 expresser children. Patients carrying *CYP2C9*1/*1* genotype are generally assumed to be extensive metabolizers; however, *CYP2C9* genotype can be converted to a phenotype different from that would be predicted from the genotype. Hence, the normal expresser children carrying *CYP2C9*1/*1* were basically expected to display extensive metabolizer phenotype, whereas low expressers with *CYP2C9*1/*1* genotype were assumed to behave as poor metabolizers. It should be noted that the mutant *CYP2C9* alleles are translated into non-functional CYP2C9 protein, resulting in poor metabolism, even if they are expressed at normal levels.

The present study, involving pediatric patients younger than 15 years of age, has clearly demonstrated that normalized serum concentrations of VPA were associated with patients' CYP2C9-status determined by *CYP2C9* genotyping and CYP2C9 expression analysis. The children with heterozygous *CYP2C9* genotype (*CYP2C9*1/*2* or *CYP2C9*1/*3*) were found to be poor VPA metabolizers, presenting high serum VPA concentrations and requiring low VPA dose. Although the patients carrying two wild type alleles (*CYP2C9*1/*1*) could be supposed to have functional CYP2C9 enzyme, their VPA metabolizing capacity was influenced by CYP2C9 expression. The low expresser patients carrying *CYP2C9*1/*1* showed as high serum VPA concentrations and required as low dose for the optimal VPA levels as those poor VPA metabolizers with heterozygous *CYP2C9* genotype, whereas the normal expressers with two wild type alleles appeared to be more active in VPA metabolism, presenting significantly lower VPA serum levels.

Phenoconversion of patients' genotype are generally explained by the fact that external or internal factors, notably co-medications, nutrition, diseases, inflammation or hormonal status, modify the expression or the function of drug metabolizing enzyme. The co-administration of VPA and antiepileptic drugs known to be CYP2C9 inducers (e.g. phenytoin, phenobarbital, or carbamazepine) results in increased CYP2C9 expression and enhanced VPA metabolizing capacity of patients on multi-drug therapy. Amini-Shirazi et al. have reported that the concomitant treatment of patients with VPA and CYP2C9 inducers increased the formation rate of 4-ene-VPA metabolite comparing to the patients on VPA monotherapy [28]. Nevertheless, the patients with distinct CYP2C9 expression occurred in both *CYP2C9*1/*1* and *CYP2C9*1/mut* genotype groups of the patients involved in our study that could not be a consequence of co-medications, because the patients on multi-drug therapy were excluded from the study. The ratio of low expresser patients was unusually high, more than half of the children involved displayed low CYP2C9 expression, predicting some suppressive factors in the background. The significant release of pro-inflammatory cytokines observed in epileptic patients following seizures seems to be a logical explanation, since the expression of drug metabolizing enzymes is down-regulated as a response to the increasing levels of the acute phase proteins, resulting in substantial impairment of drug metabolism [29-31]. The down-regulation of CYP2C9 by the pro-inflammatory cytokines, such as IL-6 and IL-1β, is proposed to be mediated by the repression of the nuclear receptors (pregnane X receptor and constitutive androstane receptor) involved in CYP2C9 expression [32,33]. The phenoconversion of other drug metabolizing enzymes, including CYP2C19, CYP2D6, CYP3A4 or NAT2, has also been observed in patients suffering from HIV, cancer or liver disease [34-38]; however, the present work was the first study that provided evidence for the phenoconversion and marked repression of VPA metabolizing CYP2C9 in epileptic children.

The novel findings of the present study demonstrated that the normalized VPA serum concentrations in pediatric patients were influenced by the patients' CYP2C9-status determined not only by the genetic variability of *CYP2C9*, but also by CYP2C9 expression. The pediatric patients with various CYP2C9-statuses required different doses of VPA for the optimal serum concentrations. The low CYP2C9 expressers and patients with mutated *CYP2C9* alleles (*CYP2C9*2* or *CYP2C9*3*) required approximately half of the dose for normal (or medium) expressers with *CYP2C9*1/*1* genotype (14-18 mg/kg *vs* 33 mg/kg). As a consequence, CYP2C9-status can guide the appropriate targeting of VPA dose at the beginning of anticonvulsant therapy. The VPA therapeutic strategy for the normal CYP2C9 expressers with *CYP2C9*1/*1* genotype can follow the conventional therapy (target VPA dose of 30-40 mg/kg) [19]. The low expressers and patients with mutated *CYP2C9* alleles (*CYP2C9*2* or *CYP2C9*3*) require substantial modification of VPA dose (14-18 mg/kg) for achieving the desired target serum concentrations. Despite the small size of genotype groups, our results would raise the concerns that the conventional clinical practice may overdose more than 70% of the pediatric patients, and *CYP2C9* genotype-controlled VPA would also increase the misdosing risk in about two third of patients carrying *CYP2C9*1/*1*. It can be concluded, that the phenoconversion of *CYP2C9* limits the predictive value of *CYP2C9* genotyping in optimizing VPA therapy.

Conclusion & future perspective

The optimal serum concentration of VPA is strongly influenced by the patients' VPA metabolizing capacity which is also critical to avoid the therapeutic failure or toxicity of VPA. Glucuronide conjugation has been demonstrated to be the major metabolic pathway of VPA in adults; however, the influence of genetic variants of UGT isoenzymes on dose-requirement and treatment outcome remains elusive because of the conflicting results obtained from small

cohort studies. According to our knowledge, CYP-mediated oxidation is not the major route of VPA metabolism in adults; however, our present work clearly demonstrated that CYP2C9 played a prominent role in children younger than 15 years of age. CYP2C9 pathway may be assumed to be more dominant in neonates and infants because of their strongly deficient glucuronidation ability, and focusing on younger pediatric patients may provide better understanding of the increased risk of VPA-induced toxicity in this vulnerable population.

Comparing to the conventional clinical practice, the *CYP2C9* genotype-based medication may bring some benefit to children on VPA therapy; however, metabolic activity of CYP2C9 is often overestimated by the prediction from the patient's *CYP2C9* genotype. The major source of overestimation is CYP2C9 phenoconversion that can be attributed to the *CYP2C9* down-regulation by cytokines in epilepsy. Thus, prospective investigation of pediatric patients' genetic and non-genetic variations in CYP2C9 allows prediction of potential 'poor metabolizers' carrying *CYP2C9* alleles with loss-of-function mutations or displaying low CYP2C9 expression. CYP2C9-status controlled medication may facilitate the improvement of the individual VPA therapy, leading to the dosage optimization for a more effective therapy, and minimizing the risk of severe side effects. Further prospective studies evaluating the clinical outcome are supposed to reveal the benefit of CYP2C9-status controlled VPA therapy over conventional antiepileptic therapy.

Executive summary

Background

- The mainstay of antiepileptic therapy is valproic acid (VPA), which is well-tolerated by most of the patients; however, the risk of serious side effects, such as hepatotoxicity or hematologic disorders, is increased in pediatric patients.

- In adults, the major metabolic pathways of VPA are glucuronidation and mitochondrial β-oxidation, whereas cytochrome P450 (CYP)-dependent oxidation has minor role in VPA metabolism.
- In children, CYP2C9-catalyzed oxidation may become the principal route of the metabolism which may lead to age-related differences in the incidence of adverse reactions.
- Although genetic polymorphism of *CYP2C9* may explain some interindividual differences in pharmacokinetics and dose-requirement of VPA, non-genetic factors give rise to low or even high CYP2C9 expression, modifying the patient's VPA metabolizing capacity.

Findings & conclusion

- *CYP2C9* genotyping of pediatric patients was able to predict VPA poor metabolism in approximately 30% of patients.
- CYP2C9 expression was down-regulated in more than 50% of children probably due to the cytokine release in epilepsy; thus, inferring the patients' VPA metabolizing phenotype merely from *CYP2C9* genotype resulted in false prediction.
- Although the VPA therapeutic strategy for the normal CYP2C9 expressers with *CYP2C9*1/*1* genotype can follow the conventional therapy (target VPA dose of 30-40 mg/kg), the low expressers and patients carrying loss-of-function mutation in *CYP2C9* gene require substantial modification of VPA dose (14-18 mg/kg) for achieving the desired target serum concentrations.
- CYP2C9-status controlled VPA therapy can contribute to the avoidance of misdosing and potential adverse reactions in pediatric patients.

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Conflict of interest & Financial disclosure

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Ethical conduct of research

CYPtesting of the patients was approved by the Hungarian Committee of Science and Research Ethics. The study was performed under the regulation of Act CLIV of 1997 on Health and of the decree 23/2002 of the Minister of Health of Hungary, and in accordance with the declaration of Helsinki. The representatives of each patient gave their informed consent to participate in this study.

545 Table 1. Demographic data of patients with various CYP2C9-statuses
546

CYP2C9-status		Number of patients	Age (year)*	Body weight (kg)*	Boys/girls
*CYP2C9*1/*1*	Normal expressers	12	4 (0.5 – 15)	25 (6 – 60)	4/8
	Low expressers	23	7 (1.5 – 15)	27 (14 – 65)	10/13
*CYP2C9*1/mut*	Normal expressers	11	4 (3 – 14)	19 (14 – 52)	5/6
	Low expressers	4	7.5 (4 -15)	22.5 (15 – 60)	1/3
Total		50	6.75 (0.5 – 15)	21.5 (6 – 65)	20/30

547 *: median (range); *CYP2C9*1/mut*: *CYP2C9*1/*2* or *CYP2C9*1/*3*
548

549

Figure legends

Figure 1. Serum concentrations of valproic acid in patients with various CYP2C9-statuses. The serum concentrations were measured four weeks after the beginning of valproic acid therapy.

*CYP2C9*1/mut*: heterozygous *CYP2C9* genotype (*CYP2C9*1/*2* or *CYP2C9*1/*3*); normal: normal (medium) CYP2C9 expressers; low: low CYP2C9 expressers; bw: body weight; *: significant difference (P<0.05); solid line: median of the groups

Figure 2. Valproic acid dose required for the therapeutic serum concentrations in patients with various CYP2C9-statuses.

*CYP2C9*1/mut*: heterozygous *CYP2C9* genotype (*CYP2C9*1/*2* or *CYP2C9*1/*3*); normal: normal (medium) CYP2C9 expresser; low: low CYP2C9 expresser; *: significant difference (P<0.05); ns: not significant

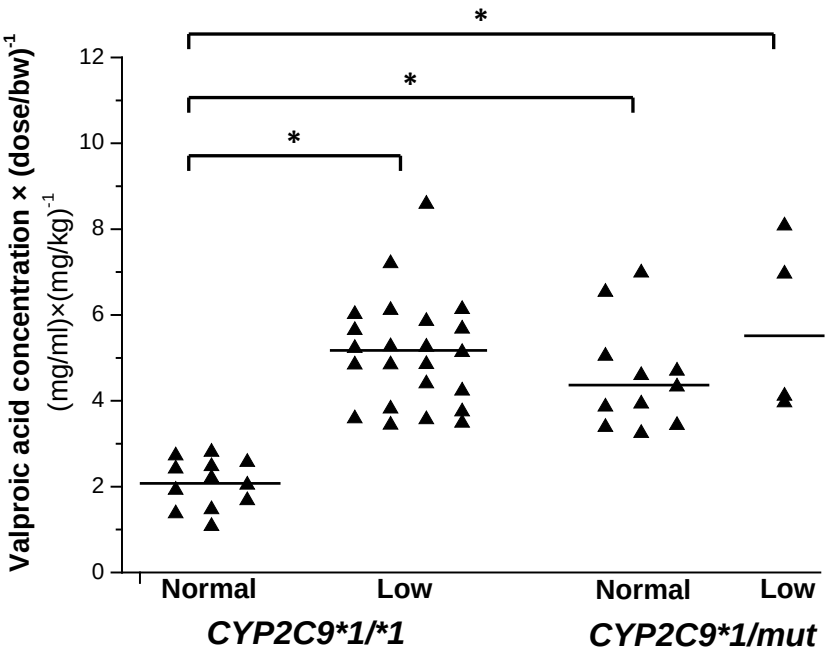
Valproic acid concentration × (dose/bw)-1
(mg/ml)×(mg/kg)-1
12
10
8
6
4
2
0
*
*
*
Normal
Low
Normal
Low
CYP2C9*1/*1
CYP2C9*1/mut

Figure 1

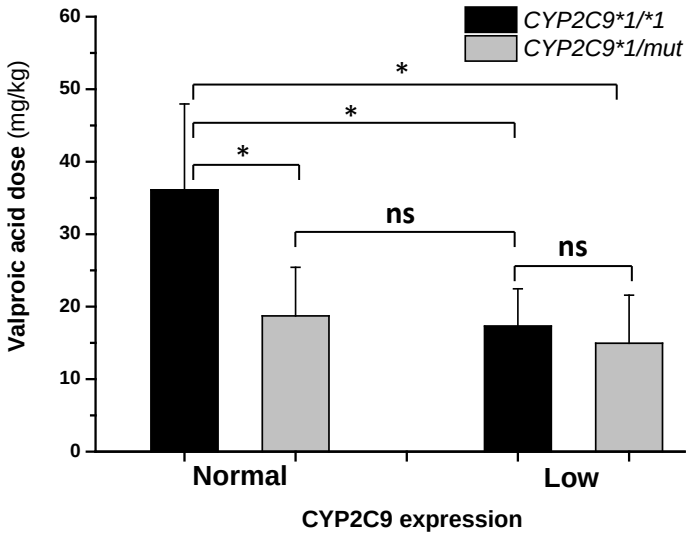
CYP2C9*1/*1
CYP2C9*1/mut
Valproic acid dose (mg/kg)
60
50
40
30
20
10
0
*
*
*
ns
ns
Normal
Low
CYP2C9 expression

Figure 2