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Association of genetic variants in *UGT1A6* genes and non-genetic variant with valproic acid doses and plasma concentration in Thai epileptic patients

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Abstract

The aim of this study was to investigate the association of genetic variants in *UGT1A6* 19T>G, 541A>G and 552A>C together with non-genetic variant with VPA dose and plasma concentrations in Thai epileptic patients. Eighty four Thai epileptic patients who were treated with VPA maintenance dose at Phramongkutklao Hospital were enrolled to this study. Three candidates single nucleotide polymorphisms (SNPs), including *UGT1A6* 19T>G, 541A>G and 552A>C were genotyped by using Taqman SNP genotyping assay. Non-genetic factor, co-medication, was divided into three categories including drug inducer, drug inhibitor and drug with no effect. Nonparametric methods (Mann-Whitney U and Kruskal-Wallis test) were used to identify the association of genetic and non-genetic variants, drug interaction, with VPA maintenance dose and steady state trough plasma concentration. The three candidate SNPs of 84 patients were genotyped. All of the genotypic distributions were consistent with Hardy-Weinberg equilibrium. For co-medication, only drug inducer and drug with no effect which were 37 and 47 respectively were found. Results of this study demonstrated that *UGT1A6* 541A>G and 552A>C variants were associated with lower VPA dose (*p*-value = 0.037, 0.031, respectively). Whereas, non-genetic factor, drug inducer tended to be associated with higher VPA dose. In line with this, drug inducer was found to be associated with lower VPA plasma concentration (*p*-value = 0.002) while *UGT1A6* polymorphisms were shown a tendency of association with higher VPA plasma level. The present study demonstrated that genetic variants in *UGT1A6* influenced variability in VPA dose while drug interaction had an impact on VPA plasma concentration in Thai epileptic patients. These finding suggested that genetic variants in gene encoding drug metabolizing enzyme and non-genetic factor, drug inducer, could explain in part the inter-individual variability in VPA maintenance dose and blood levels.

Key Words: UGT1A6, Non-genetic variants, Valproic acid, Thai epileptic patients

Introduction

Valproic acid (VPA, 2-n-propyl-pentanoic acid) is one of the most widely used antiepileptic drugs (AEDs). VPA was found to be useful in partial seizure, generalized seizure and other brain diseases such as bipolar disorder, migraine, neuropathic pain and cancer [1-5]. However, adjusting dose and monitoring blood level are necessary during the treatment owing to its narrow therapeutic range (50-100 mcg/ml) and its variability in both dose and plasma concentration in each patient [6-11]. These variabilities may originate from both genetic and non-genetic factors.

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In terms of genetic factors, variability in VPA pharmacokinetics was found to be one of the most important elements. Biotransformation of VPA is extremely complex as many enzymatic reactions are involved in VPA metabolism. Three major metabolic pathways including glucuronidation, beta-oxidation and cytochrome P450s oxidation reactions are relevant in its metabolism [12-14]. Glucuronidation, one of the important phase II biotransformation reactions, is the most extensive metabolic pathway of VPA, accounting for 50% of a dose [12-16]. It was found that UGT2B7, UGT1A6 and UGT1A3 played a principal role in the production of VPA glucuronides [15, 17-23]. Highly polymorphic genes encoding these isozymes were reported. Some of these genes could lead to both transcription and functional changes of the enzymes [24-28]. The UGT2B7 isozyme showed the highest activity in VPA glucuronidation [8,23]. However, its variants have been found to have few effects on the pharmacokinetics of many compounds, including VPA [8, 22-24, 29-33]. Three SNPs of the *UGT1A6* gene, including 19T>G, 541A>G and 552A>C, were identified. Most previous in vitro, in vivo and clinical studies of these three SNPs reported at least one SNP of these *UGT1A6* variations altered activity of enzyme comparing with the wild type [31, 34-40]. These results indicated that genetic polymorphisms in *UGT1A6* may play a role in VPA treatment. However, some recent studies reported no effect between these SNPs carriers and the non-carriers [30,41]. These contradictory and limited results lead to necessity of additional studies in order to determine the functional significance of these SNPs in epileptic patients treated with VPA.

Table 1 Demographic and clinical characteristics of the participants

Characteristics	No., (Mean ± S.D.)	%, (Range)
Total	N = 84	
Gender		
- Male	47	55.95
- Female	37	44.05
Age (years)	(41.05 ± 14.09)	(12 - 69)
Weight (kg)	(64.91 ± 16.39)	(30.0 - 109.5)
VPA dose (mg/day)	(1284.52 ± 622.81)	(200 - 4000)
VPA dose/weight (mg/day/kg)	(20.72 ± 10.75)	(3.23 - 72.73)
VPA plasma concentration (ug/ml)	(63.01 ± 31.89)	(2.00 - 179.46)
Css (ug*kg/ml*mg)	(3.63 ± 2.09)	(0.09 - 11.20)
Classification and etiology of epilepsy		
- Localized-related epilepsy	74	88.10
- Generalized epilepsy	8	9.52
- Unclassified epilepsy	2	2.38
- Special syndrome	0	0
Co-medication		
- Clonazepam	18	19.78
- Levetiracetam	13	14.29
- Phenytoin	12	13.19
- Topiramate	12	13.19
- Carbamazepine	11	12.09
- Lamotrigene	9	9.89
- Phenobarbital	8	8.79
- Oxcarbazepine	4	4.40
- Pregabalin	2	2.20
- Gabapentin	1	1.10
- Zonisamide	1	1.10
Drug interaction		
- Drug with no effect	47	55.95
- Drug inducer	37	44.05

Css : Standardized plasma trough concentration [trough plasma concentration/(daily dose/weight)]

In addition to genetic variations, non-genetic factors may also affect individual variabilities in VPA therapy. During antiepileptic drug treatment, co-medication is common depending on responsiveness of the patients. Therefore, many drug interactions between AEDs, including drug inducers and inhibitors, have been reported. At present, there are only few studies including non-genetic factors into their studies.

For these reasons, the purpose of the present study was to investigate the association of genetic variants in *UGT1A6* 19T>G, 541A>G and 552A>C together with non-genetic variants with VPA dose and plasma concentrations in Thai epileptic patients.

Methods

Patients and blood sampling. A total of 84 Thai epileptic patients were enrolled to this study during April to November 2013 at Phramongkutklao Hospital, Bangkok, Thailand. All patients were diagnosed with epilepsy and had received VPA (Depakine, Sanofi Aventis) alone or with other anticonvulsant drugs to control epilepsy.The patient was required to take at least one month of VPA to ensure that blood sampling was performed at the steady-state of VPA pharmacokinetics. Patients having liver or kidney dysfunction, a history of non-compliance, unclear medical history, drug or alcohol abuse, psychological disorders, or being pregnant or breast feeding were excluded from this study. All patient medication records and their seizure history were collected from out-patient medical records and by interviewing the patients or their caregivers. The protocol of this study was approved by the ethic committees of Phramongkutklao Hospital (approval number IRB/RTA 266/2556, issued date 18th February 2013). Written informed consent was given by all participants.

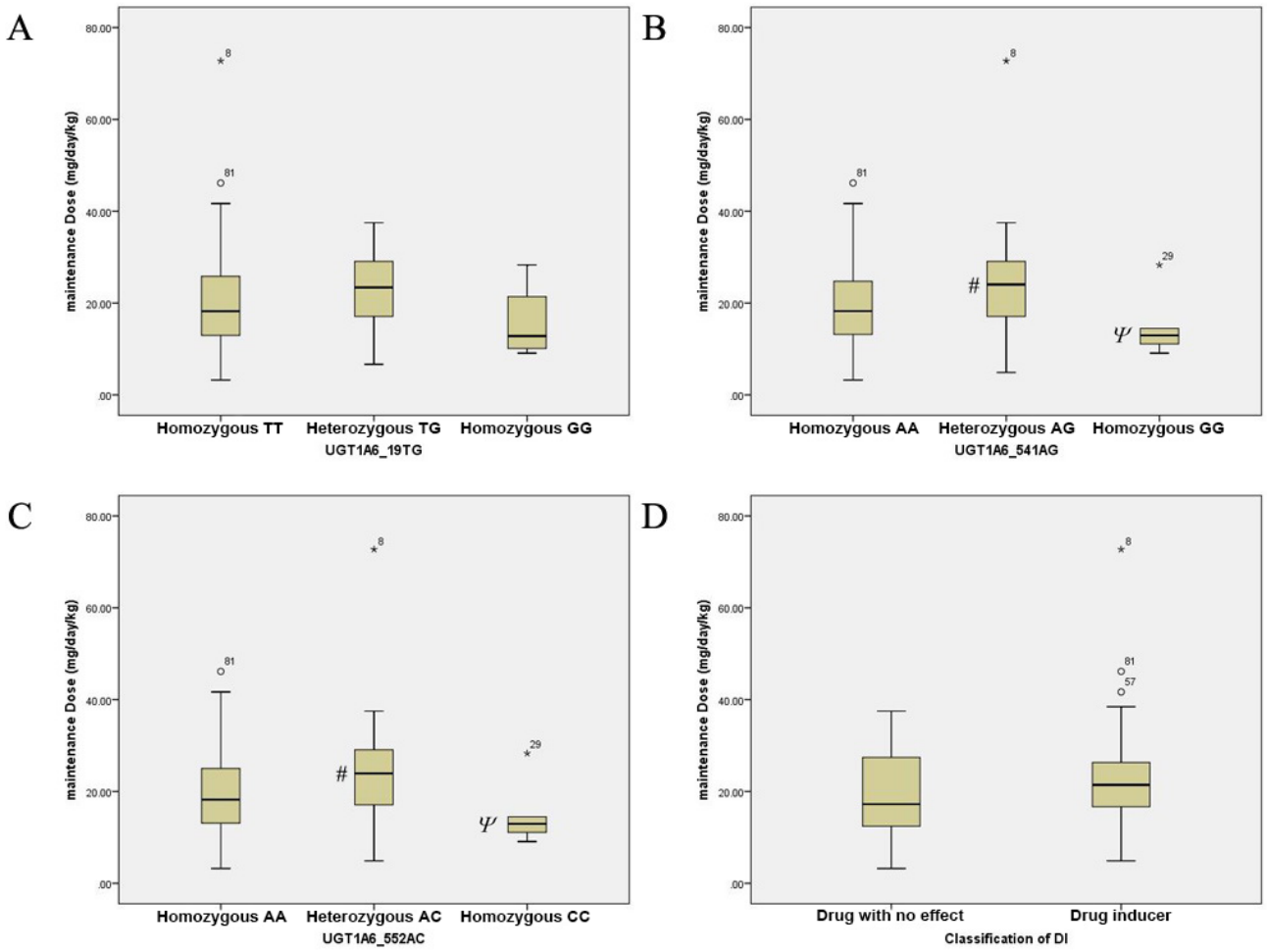

Figure 1 The relationship between *UGT1A6* 19T>G (**A**), 541A>G (**B**) , 552A>C (**C**) genetic variants, drug interaction (**D**) and VPA dose. Data were examined by using the non-parametric Kruskal-Wallis or Mann-Whitney U test followed by Bonferroni-Holm step down test, $^{\#}p < 0.05$ when compared between heterozygous variant and wild type , $^{\Psi}p < 0.05$ when compared between heterozygous and homozygous variants, *outliers

Table 2 Genotypes distribution of *UGT1A6* 19T>G, 541A>G and 552A>C genetic variants

Genotypes	Number (%)	Allele frequency	Hardy-Weinberg equilibrium P value
UGT1A6 19T>G TT TG GG	(n=84) 56 (66.67) 24 (28.57) 4 (4.76)	T = 0.81 G = 0.19	0.5004
541A>G AA AG GG	(n=84) 51 (60.71) 27 (32.14) 6 (7.14)	A = 0.77 G = 0.23	0.3672
552A>C AA AC CC	(n=84) 50 (59.52) 28 (33.33) 6 (7.14)	A = 0.76 C = 0.24	0.4565

Ten-ml blood sample was drawn from each patient before the morning dose. Each blood samples was collected into two tubes; one of which was used for measuring blood level, the other for genotyping.

Quantification of VPA plasma concentration. Steady-state trough plasma concentrations of VPA were determined by using a fluorescence polarization immunoassay (FPIA) of the Abbott TDx system, according to the instructions of the manufacturer.

Genotyping. Genomic DNA was extracted from peripheral blood leukocytes using QIAamp DNA blood mini kit R (Qiagen, German). Spectrophotometry at 260 nm was used to determine DNA concentrations. The presence of *UGT1A6* T19G, *UGT1A6* A541G and *UGT1A6* A552C were genotyped by using Taqman SNP genotyping assay (Applied Biosystems, USA).

Statistical analysis. Due to interindividual variations in VPA metabolism, VPA plasma trough concentrations were standardized by dose and body weight of each patient and expressed as Css (ug*kg/ml*mg). The data were expressed as mean ± standard deviation (SD). Hardy-Weinberg equilibrium analysis of *UGT1A6* 19T>G, 541A>G and 552A>C were examined by Chi-square test. The association of dosages and concentrations with each genetic polymorphism or drug interaction was analyzed by nonparametric methods including Kruskal-Wallis test (for comparisons of more than two groups) and Mann-Whitney U test (for two-group comparisons), as appropriate. A *p*-value of less than 0.05 was considered to be statistically significant. Statistical analysis was performed using the SPSS v. 17.0 software.

Results and Discussion

A total of 84 patients was recruited and performed genotyping. The patients' demographic and clinical characteristics were shown in Table1. According to the guidelines of the International League Against Epilepsy 1989 [23], four categories of epilepsy were applied to classify our participants (Table 1). Most of the patients were localized-related epilepsy. Following Drug Interaction Facts, drug interaction was divided into three categories including drug inducer, drug inhibitor and drug with no effect. However, in this study only drug inducer and drug with no effect which were 37 and 47 respectively were found. The prevalence of each genotype was explored and the genotypic distributions were all consistent with Hardy-Weinberg equilibrium (Table 2). The minor allele frequencies of *UGT1A6* 19T>G, 541A>G and 552A>C were 0.19, 0.23 and 0.24 respectively. There were no significant differences of patients' characteristics between patients in each genotype.

Among the three candidate SNPs, carriers of *UGT1A6* 541A>G and 552A>C allele were significantly associated with lower VPA dose (*p*-value = 0.037, 0.031, respectively, Table 3). In Figure 1, box-plots were used to illustrate the median and distribution of VPA dose among patients who had homozygous wild type, heterozygous and homozygous variants. Patients who had homozygous variants of *UGT1A6* 541A>G seemed to need a lower VPA dose than other two groups. The similar results were observed with *UGT1A6* 5552A>C allele. Although *UGT1A6* 19 T>G was detected with no significant association with VPA dose, it seemed to need a lower dose in patients carrying homozygous variants as same as *UGT1A6* 541A>G and 5552A>C alleles (Figure 1). Whereas drug inducer which was a non-genetic variant tended to be associated with higher VPA dose. In line with the dosage result, drug inducer was found to be significantly associated with lower VPA Css (*p*-value = 0.002) while *UGT1A6* 19T>G, 541A>G and 5552A>C variants showed a tendency of association with higher VPA plasma level (Figure 2, Table 4). This association indicated that patients who were homozygous of *UGT1A6* 19T>G, 541A>G and 5552A>C variants had higher VPA Css and seemed to require lower dose than other groups. Whereas, patients who received drug inducer with VPA had a tendency to need a higher dose of VPA than the patients who did not.

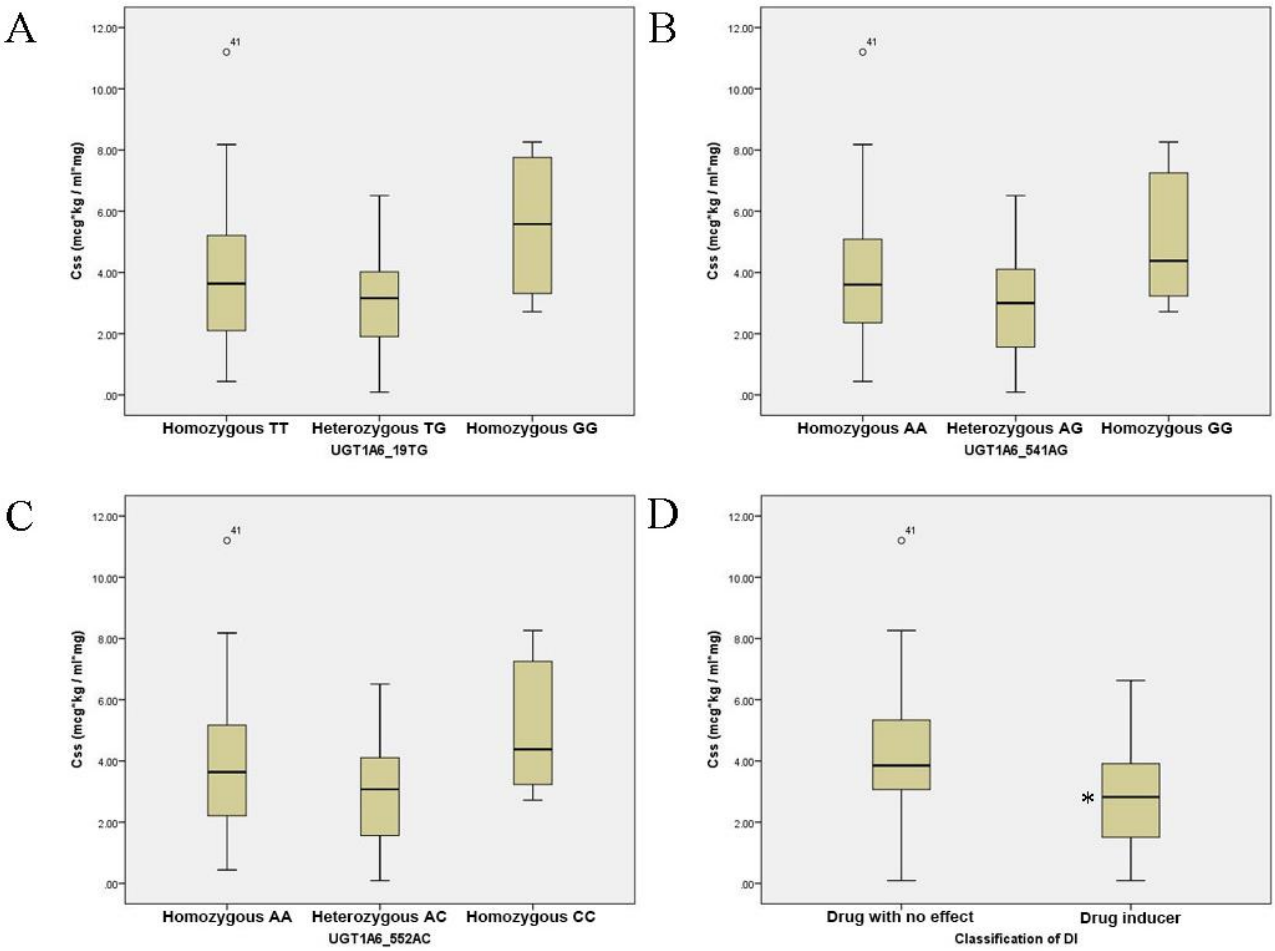

Figure 2 The relationship between UGT1A6 19T>G (**A**), 541A>G (**B**), 552A>C (**C**) genetic variants, drug interaction (**D**) and VPA Css . Data were examined by using the non-parametric Kruskal-Wallis or Mann-Whitney U test, **p* < 0.05

UGT1A6 has been previously studied and identified for many of its polymorphisms. Many studies reported the association of the polymorphisms with altered metabolic pathway of many compounds [24-28]. Additionally, changed enzyme activity of *UGT1A6* variants and variability in dose and plasma concentration of VPA have been reported [8, 19, 27, 30-31 ,33-41]. However, these results were still controversial. Few studies demonstrated UGT1A6*2 allele (*UGT1A6* 19T>G, 541A>G and 552A>C) had twice as much activity as the wild type [19,36]. In addition, homozygous allele of *UGT1A6* polymorphisms were found to have lower VPA plasma concentration than the wild type and need a higher dose of VPA to reach the therapeutic target range [31,33]. Sun et al. reported a 20% lower mean value of valproate's serum concentration in patient carrying *UGT1A6* 552A>C variant [40]. On the other hand, one of the studies reported decreased enzyme activity of *UGT1A6* polymorphims [37]. Using human liver microsomes, Peters et al. failed to find any different rate of *UGT1A6* polymorphisms [38]. Whereas, Krishnaswamy et al. found higher Km value of homozygous variants (*2/*2) than wild type [35]. In Wang et al. study, they found no influence of patients who present T181A substitution on VPA pharmacokinetics in Chinese epileptic patients [41]. In the same way, no significant differences between *UGT1A6* polymorphisms and pharmacokinetics of VPA were reported in Chu et al. study [30]. Our results revealed significant association of *UGT1A6* 541A>G and 552A>C with lower VPA dose whereas there was a tendency of association of co-medication (enzyme inducer) with higher VPA dose. In line with this, there was a significant association of enzyme inducer with lower VPA Css. A tendency of association was found between *UGT1A6* 19T>G, 541A>G, 552A>C and lower VPA Css. These results may indicate *UGT1A6* polymorphisms were related with VPA dose and tended to associate with VPA plasma concentration. However, because our study was conducted in a clinical setting, the opposed effect of non-genetic factor, which was enzyme inducer, may strongly affected and masked the association of genetic variants with VPA pharmacokinetics.

Furthermore, complexity of VPA metabolic pathways may minimize the effect of genetic variants in *UGT1A6* on VPA pharmacokinetics. Therefore, the decreasing activity of enzyme originated from genetic variants was

Table 3 Influence of *UGT1A6* 19T>G, 541A>G and 552A>C genetic variants on VPA dose and Css

Genotypes	Sample (n=84)	Dosage (mg/kg/day)	Css (µg*kg/ml*mg)
UGT1A6			
19T>G		19.95 ± 1.55	3.78 ± 0.29
TT	56	23.35 ± 1.77	2.98 ± 0.34
TG	24	15.75 ± 4.33	5.54 ± 1.32
GG	4	(p = 0.081)	(p = 0.110)
541A>G		19.38 ± 1.26	3.82 ± 0.23
AA	51	24.58 ± 2.57	2.96 ± 0.36
AG	27	14.82 ± 2.81	5.04 ± 0.92
GG	6	(*p = 0.037)	(p = 0.088)
552A>C		19.29 ± 1.28	3.84 ± 0.30
AA	50	24.55 ± 2.48	2.97 ± 0.345
AC	28	14.82 ± 2.81	5.04 ± 0.92
CC	6	(*p = 0.031)	(p = 0.080)

Data were presented as mean ± standard deviation.
Data were examined by using the non-parametric Kruskal-Wallis test, *p < 0.05
Css: Standardized plasma trough concentration [trough plasma concentration/(daily dose/weight)]

opposed by the increasing activity of enzyme in other pathways resulting from drug inducers. Consequently, the influence of genetic variants may be diminished. These results should be useful for patients taking drugs with narrow therapeutic and unpredictable blood level, which can cause problems to the patients. Because of its variable in dose and plasma concentration, the treatment goals are often found not to be accomplished.

Besides, over therapeutic range can cause toxic level or side effects to these patients. For these reasons, our research would be beneficial to providing the essential data to the further study aiming to be able to predict and adjust the dose to achieve the goals of therapy without side effect.

Conclusion

In conclusion, the present study identified genetic polymorphisms in *UGT1A6* that may explain part of the interindividual variability in VPA treatment. Two SNPs of *UGT1A6*, 541A>G and 552A>C, were found to influence VPA dosage and may lead to lower dose requirement for those homozygous variants carriers to maintain their response. On the other hand, non-genetic variant, which was drug inducers, were found to be associated with lower plasma concentration of VPA. As the results, higher dose of VPA may be required in patients having inducer drugs as their co-medication. Nevertheless, further larger studies focusing on monotherapy is required in order to support our findings.

Table 4 Influence of drug interaction on VPA dose and Css

Non-genetic variant	Sample (n=84)	Dosage (mg/kg/day)	Css (µg*kg/ml*mg)
Drug interaction			
Drug with no effect	47	18.45 ± 1.23	4.25 ± 0.32
Drug inducer	37	23.61 ± 2.08	2.84 ± 0.28
		(p = 0.061)	(*p = 0.002)

Data were presented as mean ± standard deviation.
Data were examined by using the non-parametric Mann-Whitney U test, *p < 0.05
Css : Standardized plasma trough concentration [trough plasma concentration/(daily dose/weight)]

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