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CPS1 T1405N polymorphism, HDL cholesterol, homocysteine and renal function are risk factors of VPA induced hyperammonemia among epilepsy patients

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ABSTRACT

Purpose: Valproic acid (VPA) is frequently used in the treatment of epilepsy. The adverse effects of VPA include hyperammonemia (HA) which is characterized by abnormally elevated blood ammonia level. Carbamoyl-Phosphate Synthase 1 (CPS1) is an enzyme catalyzing the initial step of removing ammonia from blood. Studies have demonstrated that the CPS1 polymorphism rs1047891-A allele carriers were susceptible to VPA-induced HA. However, the evidences remained controversial. In this study, we sought to validate the association between rs1047891 and VPA-induced HA by combining the association results from previous studies together.

Methods: We first conducted a systematic meta-analysis to determine whether rs1047891 was statistically significant. Then, we further evaluated the pleiotropic effects of rs1047891 using published genome-wide association studies (GWAS) and UKBB results. A conditional analysis was conducted to investigate whether the association between rs1047891 and VPA-induced HA was mediated by cardiovascular or renal disease risk factors or vice versa.

Results: The allelic, dominant and recessive ORs of rs1047891-A were all significant in our fixed-effect meta-analysis. In GWAS catalog and UKBB data, rs1047891 was associated with basal metabolic rate, adiposity and hematology traits, cardiovascular and renal disease risk factors. We further proved that plasma HDL cholesterol and homocysteine level, in addition to eGFR by serum creatinine, were associated with VPA-induced HA risk independently from rs1047891 polymorphism.

Conclusion: In conclusion, the SNP rs1047891 was associated with VPA-induce HA among epilepsy patients. Meanwhile, plasma HDL cholesterol and homocysteine level had independent effects from it.

1. Introduction

Valproic acid (VPA) is a broad spectrum anti-epileptic drug widely used in clinical practice and can be applied in the treatment of all kinds of epilepsies (Davis et al., 1994). Especially when violent muscle contraction is presented, VPA is the drug of the first choice (Romoli et al., 2018). However, hyperammonemia (HA) was observed in a minority of epilepsy patients treated with VPA (Gayam et al., 2018). HA is a metabolic disturbance featured by an excess of ammonia in the peripheral plasma, which may lead to the injury of brain and even death (Hernandez-Rabaza et al., 2016; Jayakumar and Norenberg, 2018). The hepatic urea cycle plays a significant role in removing ammonia from the body, in which carbamoyl-phosphate synthase 1 (CPS1) catalyzes the very first step of converting the ammonia and bicarbonate into carbamoyl phosphate (Dimski, 1994; Diez-Fernandez et al., 2015).

Genetic mutations in CPS1 gene have been reported to cause CPS1 deficiency (CPS1D, OMIM #237300) which featured HA and “valproate sensitivity”. CPS1D is an autosomal recessive disorder. Depending on the culprit mutation and/or imbalanced allelic expression, CPS1D can be early-onset which happens in infants or delayed-onset form which

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happens between early-childhood and mid-adulthood (Klaus et al., 2009; Wong et al., 1994).

Apart from the CPS1D mutations that impair urea cycle substantially, a nonsynonymous mutation, CPS1 4217C > A (rs1047891), was associated with VPA-induced HA in epilepsy patients, but the evidences have been controversial, especially in Asian population. Until now, three case-control studies were conducted in Japanese and Chinese population (Janicki et al., 2013; Inoue et al., 2014; Yagi et al., 2010). One Japanese study reported negative association between rs1047891 and VPA-induced HA, while the other two showed significant associations. The only Caucasian study combined two cohorts from the United States and Poland, which concluded that rs1047891-A was associated with increased plasma ammonia level in epilepsy patients treated with VPA (Moher et al., 2010). In addition to the controversial conclusions, we also noticed that all these studies assessed the association between rs1047891 under a dominant model, carriers (genotype AA and AC) v.s. non-carriers (genotype CC) of allele A. In this study, using data presented in these case-control studies, we evaluated the association between rs1047891 and VPA-induced HA in allelic, dominant and recessive models to better understand the relevance of this CPS1 nonsynonymous polymorphism in the pharmacogenetics of epilepsy treatment. We also investigated the possible role of the cardiovascular and renal disease risk factors in VPA-induced HA.

2. Methods

2.1. Data collection of the case-control studies

The literature search of this study was performed on 3 online databases including PubMed, EMBASE, and Web of Science. Keywords include CPS1 (or carbamoyl-phosphate synthase 1) and valproic acid induced hyperammonemia (or VPA induced hyperammonemia) and epilepsy. In order to identify additional potential studies, we also searched the list of bibliographic lists of relevant reviews and articles. The assessment of methodological quality was conducted independently by 2 reviewers, while discrepancies between reviewers were settled until a mutual agreement was reached (Fig. 1).

For the purpose of assessing the association between rs1047891 and VPA-induced HA, we only included published case-control studies with genotype counts data available. When necessary, communications with the corresponding authors were made to acquire the actual genotype counts.

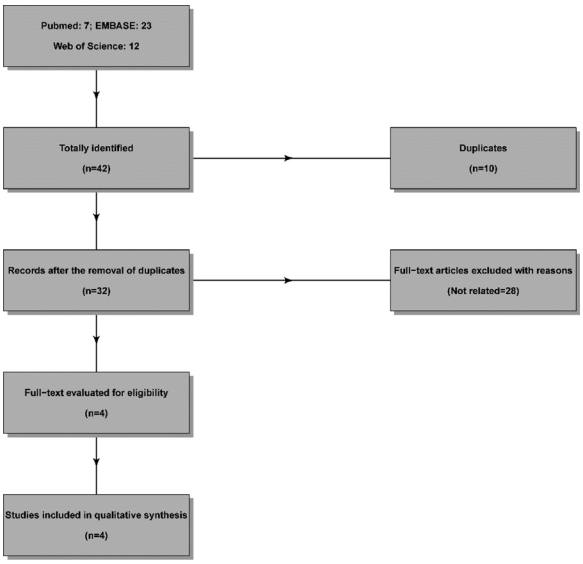

Fig. 1. Literature search flowchart.

2.2. Statistical analysis

We estimated the departure from Hardy-Weinberg Equilibrium (HWE) in control subjects using Chi-square test. Since the individual phenotype information was not available, allelic odds ratio (OR), dominant OR (OR_{dom}, AA + AC v.s. CC) and recessive OR (OR_{rec}, AA v.s. AC + CC) were calculated using 2-by-2 contingency tables. The heterogeneity among studies was assessed using Q-statistical test and I^2 test. In the case where heterogeneity among ORs of pooled studies is absent ($P > 0.05$, $I^2 < 50\%$), the fixed-effect model was applied in our meta-analysis. The sensitivity analysis where one study must be left out each time was performed by removing each study in turn and re-assessing the resulting effects on the overall effect. Egger regression test and Begg rank correlation test were adopted to evaluate the publication bias. Meta-analysis ORs were obtained by combining the ORs of studies under an inverse variance weighting (IVW) model. The data analysis and statistical testing were performed using R software (www.r-project.org) and an addon package “meta”.

2.3. Pleiotropic effects and conditional analysis

The associations between rs1047891 and disease/traits other than HA were obtained from PhenoScanner database (Staley et al., 2016). Since our study tested only one SNP rs1047891, we restricted the association p value ≤ 0.05 instead of genome-wide significance level 5×10^{-8} to detect all the possible pleiotropy effects of rs1047891. The informative pleiotropic associations with effect size, standard error, and sample size were curated into clusters depending on the relatedness of the associated traits.

We aimed to tell whether rs1047891 and the trait/disease associated with rs1047891 contributed independently to VPA-induced HA. After aligning the pleiotropic effects so that their risk alleles were A, we conducted a conditional analysis using the association summary statistics in each cluster separately, adjusted for all traits in a cluster (Deng and Pan, 2017). In each cluster, the effect sizes and SEs of rs1047891 on all traits, in addition to those of rs1047891 on VPA-induced HA, were used to estimate coefficients for each included item conditioning on the joint effect from others. In this way, we were able to identify an independent association with VPA-induced HA if the estimated coefficient of an item remained significant in the conditional analysis. Since the association summary statistics of these traits were not necessarily calculated in overlapping sample sets, we assumed there is no correlation among these traits and the null hypothesis is that HA is not associated with rs1047891 and any traits.

3. Results

3.1. Characteristics of included studies and subjects

The main characteristics of included studies were presented in Table 1. Four studies involving a total of 134 epilepsy patients with VPA-induced HA and 475 epilepsy patients showing no HA with VPA treatment were included in this study. The studies were conducted in the USA, Poland, Japan and China respectively. The allelic and genotype counts of case and control subjects were summarized in Tables 1 and 2 respectively. The control subjects of each included study were in HWE (p value > 0.05).

3.2. Meta-analysis

To assess the general association between rs1047891 and VPA-induced HA, we firstly estimated allelic OR for each included study taking allele A as the risk allele, then meta-analyzed these ORs for an overall estimation in a fixed-effect model. As shown in Table 1 and Fig. 2, allele A of rs1047891 significantly increased the risk of HA (OR = 2.37, $P = 2.0 \times 10^{-5}$). At the same time, there was no significant

Table 1
Study characteristics and allele counts.

Study	Country	Ethnicity	Sample size	Case		Control		MAF	P value
				A	C	A	C		
Yagi et al. (2010)	Japan	Asian	5/74	5	5	21	127	0.142	0.008
Inoue et al. (2014)	Japan	Asian	18/159	5	31	38	280	0.119	0.736
Zhu et al. (2018)	China	Asian	100/110	44	156	26	194	0.118	0.006
Janicki et al. (2013)	USA	Caucasian	11/132	13	9	74	190	0.280	0.004
Meta-analysis			134/475						2.0×10^{-5}

Case: epilepsy patients with VPA-induced HA; Control, epilepsy patients without VPA-induced HA; Sample size was given in (number of cases) / (number of controls); MAF, frequency of minor allele (A) in controls; P value, significance level of allelic OR.

Table 2
Genotype counts and association in dominant and recessive model.

Study	Case			Control			P_{HWE}	P_{dom}	P_{rec}
	AA	CA	CC	AA	CA	CC			
Yagi et al. (2010)	1	3	1	1	19	54	0.640	0.038	0.054
Inoue et al. (2014)	1	3	14	0	38	121	0.087	0.874	0.045
Zhu (2018)	2	40	58	2	22	86	0.671	0.002	0.923
Janicki et al. (2013)	3	7	1	10	54	68	0.873	0.026	0.043
Meta-analysis								4.0×10^{-4}	0.005

Case: epilepsy patients with VPA-induced HA; Control, epilepsy patients without VPA-induced HA; P_{HWE}, Hardy-Weinberg Equilibrium P value; P_{dom}, the P value of OR in dominant model; P_{rec}, the P value of OR in recessive model.

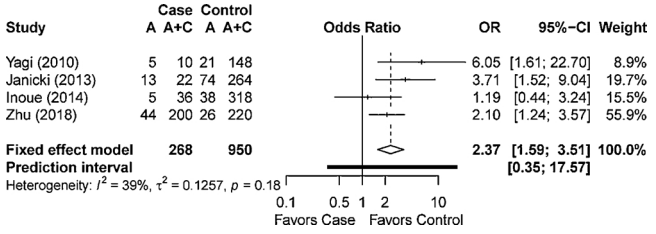

Fig. 2. Allelic association between VPA-induced HA and rs1047891.

heterogeneity among the four included studies (P = 0.18, I^2 = 39%).

We could not determine the inheritance model of VPA-induced HA associated with rs1047891-A with the included case-control data. However, a trend favorable for a recessive model, as in CPS1D, was observed. In our study, univariate OR_{dom} of each included studies were similar as compared to the original reports (Fig. 3). Although the OR_{dom} of a Japanese study seems to be on the opposite direction (P value = 0.874), the combined HA risk in allele A carriers was significantly higher than in the non-carriers (OR_{dom} = 2.49, P value = 4.0×10^{-4}). The recessive model analysis of each included study suffered the limited number of AA genotype carriers (Fig. 4). The HA risk of AA genotype was not significant in the Chinese and one of the Japanese studies, while the combined the risk throughout the included studies was significantly higher in the carriers of AA genotype than the other two genotypes (OR_{rec} = 4.41, P value = 0.005). Finally, we concluded that rs1047891-A was associated with the increased risk of HA in epilepsy patients treated with VPA and the recessive model was apparently

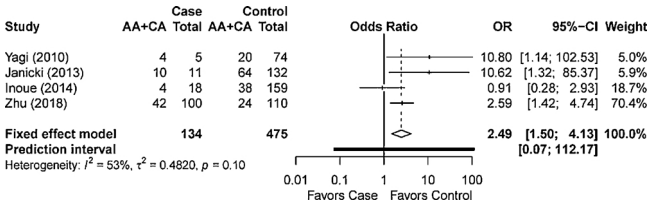

Fig. 3. Forrest plot of the association in dominant model.

stronger than the dominant model.

3.3. Sensitivity analysis and publication bias

The leave-one-out sensitivity analysis proved our meta-analysis to be reliable. The risk carried by rs1047891-A on VPA-induced HA in patients with epilepsy remained significantly higher after the exclusion of any included study (p value ≤ 0.05). The relatively symmetrical funnel plot (Fig. 5) added credits to the result. The insignificant Egger test (P = 0.693) and Begg test (P = 1.000) indicated that there was no obvious publication bias.

3.4. Pleiotropy effects and conditional analysis

Using published GWAS and UK Biobank results, we were able to identify 23 traits that were associated with rs1047891 (Supplementary Table 1). We curated these traits into 5 clusters related to adiposity, hematology, cardiovascular diseases, kidney function, and metabolic rate.

In our conditional analysis, rs1047891 remained significantly associated with HA no matter adjusted for the traits in any cluster. Moreover, high density lipoprotein cholesterol (HDLC) and homocysteine (Hcy) level were significantly associated with HA in the conditional analysis of cardiovascular traits (P value = 0.048 and 3.97×10^{-7}, respectively). Estimated glomerular filtration rate from serum creatinine level (eGFRcrea) was also significant in the conditional analysis of kidney functions cluster (P value = 4.12×10^{-3}). When taking HDLC, Hcy and eGFRcrea as covariates in the conditional analysis, we found Hcy to be associated with VPA-induced HA (P value = 0.003, beta = −0.026), while eGFRcrea and HDLC became insignificant. As a result, our conditional analysis not only revealed consistent association between rs1047891 and VPA-induced HA, but also identified independent risk factors which may contribute to VPA-induced HA in epilepsy patients.

4. Discussion

4.1. Possible recessive rs1047891-A allele and VPA-induced HA

In this study, we confirmed the association between CPS1 non-synonymous polymorphism rs1047891 and HA in epilepsy patients treated with VPA. Under a recessive model that was not covered by previous reports, our results demonstrated an apparently stronger effect as compared to the one seen under dominate effect (OR_{rec} = 4.41, OR_{dom} = 2.49).

HA, as a major symptom of CPS1D, may occur when the dysfunction of CPS1 caused slower ammonia removal from blood. In the case of CPS1D, people with homozygous or compound heterozygous of the defective alleles catch with HA. In clinical practices, HA was evident when VPA was used to treat epilepsy patients without CPS1D mutations and rs1047891-A, which was probably caused by the accumulation of VPA metabolites, such as 2-propyl-4-pentenoic acid, propionate, and

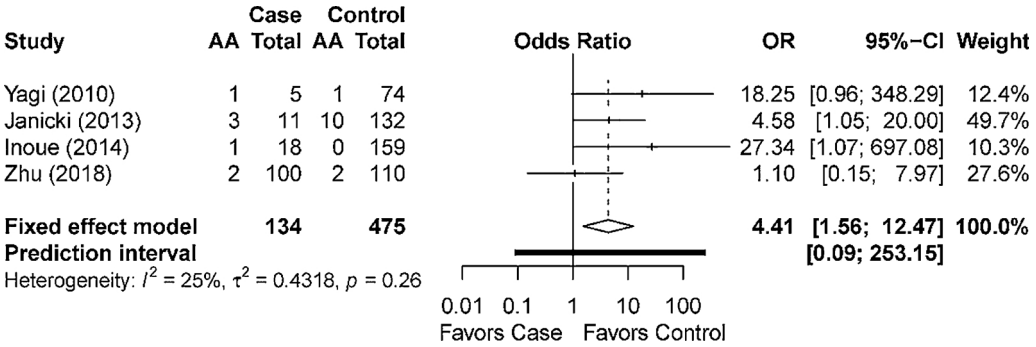

Fig. 4. Forrest plot of the association in recessive model.

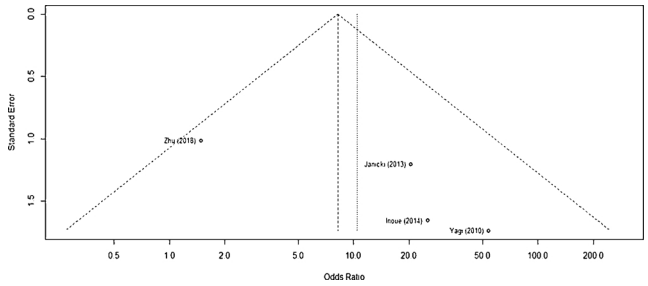

Fig. 5. Funnel plot for our meta-analysis.

valproic-CoA (Nanau and Neuman, 2013). Hence, the effect of rs1047891-A and VPA can be overlapped, resulting even elevated blood ammonia level.

According to the consequence predictions by PolyPhen (Adzhubei et al., 2010) and SIFT (Ng and Henikoff, 2003), the threonine to asparagine transition introduced by rs1047891 was benign or tolerated. In addition, rs1047891-A allele is common in world populations, ranging from 14% in East Asians to 37% in African population. Therefore, we hypothesize that rs1047891-A might be a mild modifier of CPS1 enzyme activity which increased the susceptibility of HA and contributed to the adverse effect of VPA. This hypothesis was also supported by a GWAS in which another CPS1 polymorphism (rs715) in strong linkage disequilibrium (LD) with rs1047891 was associated with metabolites from the urea cycle (Hartiala et al., 2016). Since the two polymorphisms are in high LD, we would expect rs1047891 to be associated with metabolites from urea cycle which CPS1 plays an important role in. At present, it is unclear whether rs715, rs1047891 or another functional mutation was responsible for the elevated serum ammonia level.

On the other hand, VPA might act with CoA and decrease the availability of acetyl-CoA, which is a coactivator of CPS1 (Romshe et al., 1981; Eadie et al., 1988). As we have pointed out above, the CPS1 rs1047891-A allele or the unknown mutation responsible for this mild change in enzyme activity, may not be able to alter CPS1 activity substantially on its own. However, it is possible that the mutant CPS1 fails to continue the urea cycle because the concentration of free CoA fell below its minimum obligate concentration, which might be higher than that for normal CPS1.

4.2. The interplay among CPS1 rs1047891, Hcy and VPA-induced HA

As evident in our conditional analysis, the molecular mechanism of VPA-induced HA might be strongly linked with the metabolites that could cause damage to heart and central nervous system. In our results, plasma Hcy level, with the adjustment of rs1047891, HDLC level and eGFRcrea, was associated with VPA-induced HA with a protective effect (beta = −0.026). Hcy has been a well-known risk factor of heart diseases (Homocysteine Studies, 2002; Humphrey et al., 2008; Casas et al., 2005) and neurodegenerative diseases (Lipton et al., 1997; Kim, 2014). Higher Hcy level was also associated with rs1047891-A in European individuals and Filipino females free of VPA treatment (Lange et al., 2010; van Meurs et al., 2013). On the other hand, plasma Hcy level and anomia level are correlated. Hcy is catabolized into cysteine and α-ketobutyrate through the transsulfuration pathway, producing ammonia at the same time (Stipanuk, 2004). As a result, increased Hcy level and ammonia level can be both seen in liver dysfunction (Garcia-Tevijano et al., 2001; Bosy-Westphal et al., 2003; Lu et al., 2002) and chronic VPA treatment (Ni et al., 2014; Segura-Bruna et al., 2006). Taken together, our conditional analysis result may be indicating that the high plasma Hcy (or HDLC) level of epilepsy patients treated with VPA may be mainly driven by the modification effect of rs1047891 on CPS1 activity. In addition, the protective effect of Hcy on VPA-induced HA could be explained by the accumulation of ammonia in liver cells, which is able to decrease the rate of enzymatic reactions to catabolize Hcy. However, this indication should be validated using individual level data from a study with the measurement of both plasma Hcy and ammonia under VPA treatment.

4.3. The clinical utility of CPS1 rs1047891

Our study also showed a relatively weak but population-specific evidence for rs1047891 to be used in clinical application. We constructed a genetic risk score using merely allele-A dosage of rs1047891. The average genetic risk score of patients with VPA-induced HA was greater than those without (0.17, p value = 0.0042). Furthermore, we set the diagnostic cutoff as one or two copies of A allele and evaluated the predictive power of this genetic risk score. The diagnostic cutoff of two-copy (0 + 1 vs. 2) outperformed that of one-copy (0 vs. 1 + 2) in both positive predictive value (PPV = 0.35 and 0.29, for two-copy and one-copy, respectively.) and negative predictive value (NPV = 0.78 and 0.18). Thus, the diagnosis of better VPA safety (HA-free) for people not carrying two copies of rs1047891-A allele appeared to be more feasible in clinical application. Moreover, the negative and positive predictive values were substantially different in Europeans and Asians. Using two-copy cutoff, this SNP had a NPV of 0.99 in Europeans, but 0.78 in the Asians. VPA-induced HA is a polygenic adverse drug reaction which involved a complex biochemistry mechanism. Hence, more risk factors have to been taken into consideration for a precise prediction of VPA-induced HA. Last but not least, the estimated predictive power of rs1047891 in the current study may be biased by the limited sample size. Cautions have to be taken when interpreting this result.

4.4. The strength and limitation of our study

Our study explored the recessive effect of rs1047891-A on VPA-induced HA which was not considered in previous studies. The results improved the understanding of the molecular mechanism underlying VPA-induced HA. Meanwhile, our study has some limitations. Firstly, our study did not included patients of African ethnicity, in which

epilepsy incidence was higher than the global average, and the carriers of rs1047891-A were more frequent. A case-control study conducted in African population would be more informative. Secondly, the previous case-control studies defined HA using different plasma ammonia levels. Thus, the VPA-induced HA patients in the studies using more stringent definition will be more severe than the ones in the other two study. This could increase the heterogeneity in our meta-analysis. Finally, our study evaluated the association between CPS1 rs1047891 SNP and VPA-induced HA, while the functional impact of this nonsynonymous SNP on the enzyme activity was largely unknown. Further fine mapping study and functional experiments in animal models are warrant to discover the relevance of this SNP in personalized clinical practices.

Author contribution statement

L.C. and P.C. designed this research; H.Y., H.L. and C.L. did the data acquisition; P.C., Q.T., X.T. and L.C. conducted the statistical analyses. C.L., H.Y., H.L. and L.C. wrote the first draft of the manuscript; Q.T., X.G., J.W., D.C., M.Z., Y.L., X.T. and P.C. revised the manuscript; All authors agreed with the manuscript submission; P.C. and X.T. gave the final approval for the manuscript submission.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eplepsyres.2019.05.010.

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