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Blood Levels of Ammonia and Carnitine in Patients Treated with Valproic Acid: A Meta-analysis

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Objective: Long-term valproic acid (VPA) administration is associated with adverse metabolic effects, including hyperammonemia and hypocarnitinemia. However, the pathogeneses of these adverse events remain unclear, and not enough reviews have been performed. The aim of this study was to conduct a meta-analysis of studies examining blood levels of ammonia and carnitine in patients treated with VPA.
Methods: We conducted database searches (PubMed, Web of Science) to identify studies examining blood levels of ammonia and carnitine in patients treated with VPA. A meta-analysis was performed to conduct pre- and post-VPA treatment comparisons, cross-sectional comparisons between groups with and without VPA use, and estimations of the standardized correlations between blood levels of ammonia, carnitine, and VPA.
Results: According to the cross-sectional comparisons, the blood ammonia level in the VPA group was significantly higher than that in the non-VPA group. Compared to that in the non-VPA group, the blood carnitine level in the VPA group was significantly lower. In the meta-analysis of correlation coefficients, the blood VPA level was moderately correlated with blood ammonia and blood free carnitine levels in the random effects model. Furthermore, the blood ammonia level was moderately correlated with the blood free carnitine level.
Conclusion: Although the correlation between ammonia and free carnitine levels in blood was significant, the moderate strength of the correlation does not allow clinicians to infer free carnitine levels from the results of ammonia levels. Clinicians should measure both blood ammonia and free carnitine levels, especially in patients receiving high dosages of VPA.

KEY WORDS: Bipolar disorder; Valproic acid; Free carnitine; Acylcarnitine; Ammonia.

INTRODUCTION

Valproic acid (VPA) is commonly used for the treatment of psychiatric or neurological diseases. The mechanism of VPA is not fully understood, although the regulation of glutamate excitatory neurotransmission and/or gamma aminobutyric acid (GABA) inhibitory neurotransmission has been postulated [1]. While VPA is usually tolerated, adverse metabolic effects, such as hypocarnitinemia as well as hyperammonemia, have been associated with long-term VPA administration [2].

Carnitine is essential for the transport of long-chain fatty acids into mitochondria for beta-oxidation. When carnitine is lacking, fatty acids accumulate and inhibit the urea cycle via multiple pathways, resulting in elevated ammonia [3,4]. A recent meta-analysis indicated that carnitine supplementation significantly reduces blood levels of ammonia [5]. Although the abovementioned mechanisms suggest that carnitine deficiency could promote VPA-induced hyperammonemia, previous studies conducted in participants receiving VPA reported inconsistent results regarding the relationship between ammonia and carnitine [2,3,6,7]. Clarifying the relationship between ammonia and carnitine could be important for clinicians to decide monitoring plans for patients taking VPA.

Therefore, we conducted a meta-analysis of studies evaluating blood levels of ammonia and carnitine in patients treated with VPA. We aimed to (1) clarify the mean differences in ammonia and carnitine levels between patients with and without VPA treatment (cross-sectional

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comparisons), (2) describe the mean differences in ammonia and carnitine levels after VPA treatment (pre- and post-VPA comparisons), and (3) estimate the standardized correlations between blood levels of ammonia, carnitine, and VPA (meta-correlational analyses).

METHODS

Study Selection

The systematic review was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) standards (a protocol used to evaluate systematic reviews) [8]. Electronic databases, including PubMed and Web of Science, were initially searched using six terms. The search phrases for PubMed were "(valproic acid [ALL] OR valproate [ALL] OR divalproex [ALL]) AND carnitine [ALL])" OR "(valproic acid [ALL] OR valproate [ALL] OR divalproex [ALL]) AND (ammonia [ALL] OR hyperammonemia [ALL])". We used comparable search terms for Web of Science.

We included studies that had ≥ 10 participants with VPA use, regardless of clinical setting (inpatient, outpatient); (1) observational studies (cross-sectional, longitudinal studies), (2) randomized controlled trials, and (3) case reports. We excluded the following: (1) comments, editorials, letters; (2) studies not performed in human participants; (3) non-English publications; (4) studies including conditions likely to significantly affect the distribution

Table 1. Major characteristics of studies included for cross-sectional comparison

Author	Group	Unit	Mean ± SD	Number	Mean ± SD	Number	Mean ± SD	Number
Maldonado *et al.* [9], 2016	With VPA	μg/dl	105.2 ± 57.2	28				
	Without VPA		61.7 ± 27.3	31	82.1 ± 35.6	41		
Yamamoto *et al.* [10], 2013	With VPA	μg/dl	85.8 ± 42.7	1,826				
	Without VPA		36.0 ± 21.1	445	56.0 ± 28.5	673		
Castro-Gago *et al.* [11], 2010	With VPA	μmol/L	39.8 ± 14.1	57				
	Without VPA		29.5 ± 10.5	75	29.9 ± 8.1	17		
Agarwal *et al.* [12], 2009	With VPA	μg/dl	86.4 ± 39.9	100				
	Without VPA		68.7 ± 30.1	100				
Hamed and Abdella [6], 2009	With VPA	μg/dl	75.6 ± 18.0	60				
	Without VPA		36.4 ± 10.8	40				
Verrotti *et al.* [13], 1999	With VPA	μg/dl	36.7 ± 12.4	32	59.9 ± 16.3	28		
	Without VPA		31.1 ± 14.7	24	29.7 ± 12.1	40		
Hirose *et al.* [14], 1998	With VPA	μmol/L	26.0 ± 9.2	45				
	Without VPA		29.4 ± 11.8	45				
Altunbaşak *et al.* [15], 1997	With VPA	μg/dl	29.8 ± 14.6	44	32.0 ± 19.4	24		
	Without VPA		21.6 ± 20.4	16				
Thom *et al.* [16], 1991	With VPA	μmol/L	32.0 ± 24.3	37				
	Without VPA		21.0 ± 18.8	22				
Beghi *et al.* [17], 1990	With VPA	μg/dl	62.5 ± 40.9	55	56.1 ± 32.6	54		
	Without VPA		49.4 ± 31.3	51	36.5 ± 24.6	53		
Komatsu *et al.* [18], 1987	With VPA	μg/dl	39.9 ± 13.6	8	61.7 ± 24.1	25	121.9 ± 48.6	31
	Without VPA		39.3 ± 12.5	12	48.6 ± 13.2	16	39.3 ± 9.9	13
			48.1 ± 17.6	17	68.9 ± 20.0	15		
Kugoh *et al.* [19], 1986	With VPA	μg/dl	40.5 ± 23.3	53	56.6 ± 26.5	140		
	Without VPA		40.7 ± 15.2	63				
Farrell *et al.* [20], 1986	With VPA	μmol/L	30.2 ± 9.3	31	34.9 ± 9.0	19		
	Without VPA		29.8 ± 10.8	25				
Ratnaike *et al.* [21], 1986	With VPA	μmol/L	37.1 ± 31.8	23	37.6 ± 21.4	33		
	Without VPA		21.5 ± 7.8	25				
Haidukewych *et al.* [22], 1985	With VPA	μg/ml	0.8 ± 0.5	33	0.6 ± 0.3	27	0.6 ± 0.2	13
			0.3 ± 0.2	14	0.3 ± 0.2	38		
	Without VPA		0.5 ± 0.1	32				
Ohtani *et al.* [23], 1982	With VPA	μg/dl	143.8 ± 42.4	14				
	Without VPA		55.1 ± 15.0	11	46.7 ± 72.2	27		

Mean ± standard deviation (SD) of blood ammonia levels.
VPA, valproic acid.

Table 2. Major characteristics of studies included for cross-sectional comparison

Author	Group	Unit	Mean ± SD	Number	Mean ± SD	Number	Mean ± SD	Number
Qiliang *et al.* [24], 2018	With VPA	μmol/L	23.9 ± 10.6	299				
	Without VPA		36.4 ± 9.4	299				
Maldonado *et al.* [9], 2016	With VPA	μmol/L	39.8 ± 13.0	28				
	Without VPA		37.8 ± 8.6	31	50.1 ± 18.9	41		
Cansu *et al.* [25], 2011	With VPA	μmol/L	29.6 ± 7.1	28				
	Without VPA		30.9 ± 10.1	28				
Nakajima *et al.* [7], 2011	With VPA	μmol/L	40.8 ± 11.0	28	32.1 ± 8.4	23		
	Without VPA		47.7 ± 9.1	23				
Hamed and Abdella [6], 2009	With VPA	μmol/L	25.3 ± 8.1	60				
	Without VPA		40.9 ± 4.8	40				
Anil *et al.* [26], 2009	With VPA	μmol/L	16.5 ± 10.2	50				
	Without VPA		44.6 ± 7.3	30				
Zelnik *et al.* [27], 2008	With VPA	μg/ml	26.9 ± 8.6	18				
	Without VPA		38.5 ± 7.8	24	37.2 ± 7.8	28	40.4 ± 8.7	21
Werner *et al.* [28], 2007	With VPA	μmol/L	44.4 ± 10.8	16	41.1 ± 11.5	9		
	Without VPA		48.7 ± 22.1	15	47.9 ± 9.5	27		
Verrotti *et al.* [13], 1999	With VPA	μmol/L	28.9 ± 5.1	32	25.7 ± 4.3	28		
	Without VPA		40.9 ± 7.1	24	42.9 ± 8.0	40		
Castro-Gago *et al.* [29], 1998	With VPA	μmol/L	25.8 ± 6.1	17				
	Without VPA		34.3 ± 8.3	10	27.8 ± 4.4	5	49.0 ± 5.9	71
Hirose *et al.* [14], 1998	With VPA	μmol/L	42.7 ± 9.9	45				
	Without VPA		44.4 ± 9.9	45				
Hiraoka *et al.* [30], 1997	With VPA	μmol/L	35.6 ± 13.5	9	24.6 ± 5.2	13		
	Without VPA		42.7 ± 9.3	12				
Zelnik *et al.* [31], 1995	With VPA	μmol/L	29.1 ± 6.2	15				
	Without VPA		38.9 ± 14.6	14	37.2 ± 7.9	8	40.3 ± 12.8	34
Riva *et al.* [32], 1993	With VPA	μmol/L	35.0 ± 13.0	22				
	Without VPA		48.0 ± 20.0	16				
Hug *et al.* [33], 1991	With VPA	μmol/L	27.0 ± 10.0	53	23.2 ± 9.3	18		
	Without VPA		42.5 ± 14.1	32	24.6 ± 12.3	119	31.4 ± 10.4	92
			33.0 ± 8.3	141	24.0 ± 10.7	19	30.9 ± 11.0	17
			38.8 ± 10.7	12				
Thom *et al.* [16], 1991	With VPA	μmol/L	30.8 ± 10.9	37				
	Without VPA		39.3 ± 6.6	22				
Opala *et al.* [34], 1991	With VPA	μmol/L	29.9 ± 10.0	43	21.4 ± 12.0	91		
	Without VPA		36.7 ± 10.0	43	36.8 ± 7.0	89		
Matsumoto *et al.* [35], 1990	With VPA	μmol/L	44.7 ± 30.1	198				
	Without VPA		53.4 ± 20.6	50				
Beghi *et al.* [17], 1990	With VPA	μmol/L	33.0 ± 11.7	55	36.2 ± 10.4	54		
	Without VPA		37.0 ± 9.4	51	41.4 ± 8.9	53		
Melegh *et al.* [36], 1990	With VPA	μmol/L	26.1 ± 7.1	10				
	Without VPA		42.7 ± 6.8	10				
Rodriguez-Segade *et al.* [37], 1989	With VPA	μmol/L	26.4 ± 8.4	34				
	Without VPA		41.2 ± 11.7	149	42.1 ± 10.0	26	47.1 ± 7.7	49
Komatsu *et al.* [18], 1987	With VPA	μmol/L	55.7 ± 8.6	11	42.5 ± 9.5	25	36.6 ± 11.5	25
	Without VPA		57.3 ± 7.7	7	51.3 ± 13.5	7	48.5 ± 11.2	26
			53.2 ± 7.9	12	52.8 ± 17.4	5		
Melegh *et al.* [38], 1987	With VPA	μmol/L	16.8 ± 5.9	11				
	Without VPA		26.5 ± 7.0	11				
Morita *et al.* [39], 1986	With VPA	μmol/L	21.5 ± 7.4	12				
	Without VPA		31.5 ± 7.7	13	51.7 ± 8.8	32		
Laub *et al.* [40], 1986	With VPA	μmol/L	33.5 ± 8.0	21				
	Without VPA		41.2 ± 12.0	21	39.9 ± 9.0	21		
Ohtani *et al.* [23], 1982	With VPA	μmol/L	28.6 ± 9.7	14				
	Without VPA		43.0 ± 8.6	11	44.2 ± 63.3	27		

Mean ± standard deviation (SD) of blood free carnitine levels.
VPA, valproic acid.

of ammonia or carnitine levels (e.g., participants with valproate-induced hyperammonemic encephalopathy, carnitine palmitoyltransferase deficiency, hepatitis, or liver failure); and (6) studies including participants who used VPA for less than 1 month. Two researchers (SY and NS) independently searched the literature. After all papers had been assessed, any discrepancies in the responses were identified and discussed until consensus was reached.

Data Extraction

The following data were extracted: first author's name, publication year, sample size, means and standard deviation (SD) values of blood ammonia and free carnitine levels in each group, and correlation coefficients between blood levels of ammonia, carnitine, and VPA among participants taking VPA (Tables 1−4) [9-54]. Subjects whose mean levels of ammonia or carnitine were more than twice as high as the upper limit of the normal range were excluded from the final analysis.

Statistical Analysis

We calculated the mean (SD) as a one group, when there were two or more groups taking VPA in one article. Additionally, all non-VPA groups in one article were considered a single group for data synthesis purposes.

For the cross-sectional comparison, we calculated the standardized mean differences (SMDs) between the groups using the metacont function in the meta package with the option for SMD (sm = "SMD").

Regarding the pre- and post-VPA comparison, most studies included only the mean and SD of each pre- and postvisit, not the mean and SD of the difference from baseline. Therefore, we calculated the mean and SD of the differences from baseline for such studies under the assumption that the correlations between pre- and post-variables were equivalent to 0.5. We calculated the mean differences from baseline visit data using the metamean function in the meta package of R software with the default settings [55].

For the meta-correlational analysis, we transformed Spearman's correlation coefficients to Pearson's coefficients using transformation functions on the assumption that the variables followed a normal distribution after applying an adequate statistical transformation (e.g., Box-Cox transformation) [56]. We synthesized the correlations between the variables using the metacor function in the meta package with the default settings.

All meta-analyses were conducted using random effect

Table 3. Major characteristics of studies included for pre-post comparison

Author	Variables	Group	Unit	Mean ± SD	Number	Mean ± SD	Number
Hamed and Abdella [6], 2009	Ammonia	Before VPA	μg/dl	40.7 ± 5.4	60		
		After VPA		75.6 ± 18.0			
Redden *et al.* [41], 2009	Ammonia	Before VPA	μmol/L	39.2	193		
		Mean difference		11.7 ± 21.3			
Paganini *et al.* [42], 1984	Ammonia	Before VPA	μg/dl	39.1 ± 16.0	21		
		After VPA		57.6 ± 16.0			
Cansu *et al.* [25], 2011	Free carnitine	Before VPA	μmol/L	32.9 ± 10.9	28		
		After VPA		29.6 ± 7.1			
Hamed and Abdella [6], 2009	Free carnitine	Before VPA	μmol/L	36.9 ± 4.0	60		
		After VPA		25.3 ± 8.1			
Werner *et al.* [28], 2007	Free carnitine	Before VPA	μmol/L	46.5 ± 8.5	16	47.4 ± 11.7	9
		After VPA		44.4 ± 11.2		41.1 ± 11.5	
Castro-Gago *et al.* [29], 1998	Free carnitine	Before VPA	μmol/L	34.4 ± 8.5	17		
		After VPA		25.8 ± 6.1			
Van Wouwe [43], 1995	Free carnitine	Before VPA	μmol/L	32.7 ± 7.3	13		
		After VPA		20.9 ± 5.2			
Zelnik *et al.* [31], 1995	Free carnitine	Before VPA	μmol/L	37.6 ± 24.0	15		
		After VPA		29.1 ± 6.2			
Riva *et al.* [32], 1993	Free carnitine	Before VPA	μmol/L	49.0 ± 17.0	22		
		After VPA		35.0 ± 13.0			

Mean ± standard deviation (SD) of blood ammonia and free carnitine levels.
VPA, valproic acid.

Table 4. Major characteristics of studies included for meta-correlational analysis

Author	Variables	Correlational coefficient		Number
Yokoyama *et al.* [20], 2020	VPA, ammonia	0.149	Pearson	182
Duman *et al.* [44], 2019	VPA, ammonia	0.207	Pearson	94
Maldonado *et al.* [9], 2016	VPA, ammonia	0.683	Pearson	28
Günaydin *et al.* [45], 2014	VPA, ammonia	0.742	Spearman	26
Tseng *et al.* [46], 2014	VPA, ammonia	0.210	Pearson	158
Sharma *et al.* [47], 2011	VPA, ammonia	0.820	Spearman	63
Castro-Gago *et al.* [11], 2010	VPA, ammonia	0.449	Spearman	57
Moreno *et al.* [48], 2005	VPA, ammonia	0.272	Pearson	29
Verrotti *et al.* [13], 1999	VPA, ammonia	0.410	Pearson	60
Altunbaşak *et al.* [15], 1997	VPA, ammonia	0.458	Pearson	68
Patsalos *et al.* [49], 1993	VPA, ammonia	0.080	Pearson	82
Kondo *et al.* [50], 1992	VPA, ammonia	−0.233	Spearman	53
Kugoh *et al.* [19], 1986	VPA, ammonia	0.570	Pearson	53
Laub [51], 1986	VPA, ammonia	−0.362	Pearson	10
Haidukewych *et al.* [22], 1985	VPA, ammonia	0.249	Pearson	125
Williams *et al.* [52], 1984	VPA, ammonia	0.054	Pearson	10
Yokoyama *et al.* [20], 2020	VPA, free carnitine	−0.194	Pearson	182
Maldonado *et al.* [9], 2016	VPA, free carnitine	−0.616	Pearson	28
Anil *et al.* [26], 2009	VPA, free carnitine	0.180	Pearson	50
Moreno *et al.* [48], 2005	VPA, free carnitine	−0.301	Pearson	29
Hirose *et al.* [14], 1998	VPA, free carnitine	−0.410	Pearson	45
Morita *et al.* [39], 1986	VPA, free carnitine	−0.421	Pearson	12
Laub [51], 1986	VPA, free carnitine	0.097	Pearson	21
Yokoyama *et al.* [20], 2020	Ammonia, free carnitine	−0.097	Pearson	182
Okumura *et al.* [4], 2019	Ammonia, free carnitine	−0.392	Pearson	49
Ando *et al.* [53], 2017	Ammonia, free carnitine	0.020	Pearson	37
Nakajima *et al.* [7], 2011	Ammonia, free carnitine	−0.546	Spearman	51
Hamed and Abdella [6], 2009	Ammonia, free carnitine	−0.935	Pearson	60
Goto *et al.* [54], 2008	Ammonia, free carnitine	−0.420	Pearson	60
Laub [51], 1986	Ammonia, free carnitine	0.013	Pearson	21

VPA, valproic acid.

models, and the heterogeneity for each analysis result was evaluated with I-square statistic.

RESULTS

After excluding duplicates and nonrelevant studies, our search yielded 50 publications that fulfilled the inclusion criteria (Fig. 1). In the cross-sectional comparison, the blood ammonia level in the VPA group was significantly higher than that in the non-VPA group (n = 16, n = 4,821, SMD = 0.7, confidence interval [CI]: 0.5, 1.0, $p < 0.01$; I^2 = 88%) (Fig. 2). Compared to that in the non-VPA group, the blood carnitine level in the VPA group was significantly lower (n = 26, n = 3,505, SMD = −1.1, CI: −1.4, −0.8, $p < 0.01$; I^2 = 90%) (Fig. 3).

According to the pre- and post-VPA comparison, VPA treatment significantly increased the blood ammonia level (n = 3, n = 274, MRAW = 14.3 micromol/L, CI: 8.3, 20.4, $p < 0.01$; I^2 = 96%) (Fig. 4) and significantly decreased the blood carnitine level (n = 7, n = 180, MRAW = −8.7 micromol/L, CI: −11.4, −5.9, $p < 0.01$; I^2 = 79%) (Fig. 5).

The correlation coefficient between VPA and blood ammonia level was 0.36 (CI: 0.20, 0.50) (n = 16, n = 1,098, $p < 0.01$; I^2 = 86%) in the random effects model (Fig. 6). Under the same analytical conditions, the correlation coefficient between VPA and free carnitine in blood was −0.24 (CI: −0.43, −0.03) (n = 7, n = 367, $p < 0.01$; I^2 = 67%) (Fig. 7), and the correlation coefficient between ammonia and free carnitine in blood was −0.44 (CI: −0.73, −0.02) (n = 7, n = 460, $p < 0.01$; I^2 = 95%) (Fig. 8).

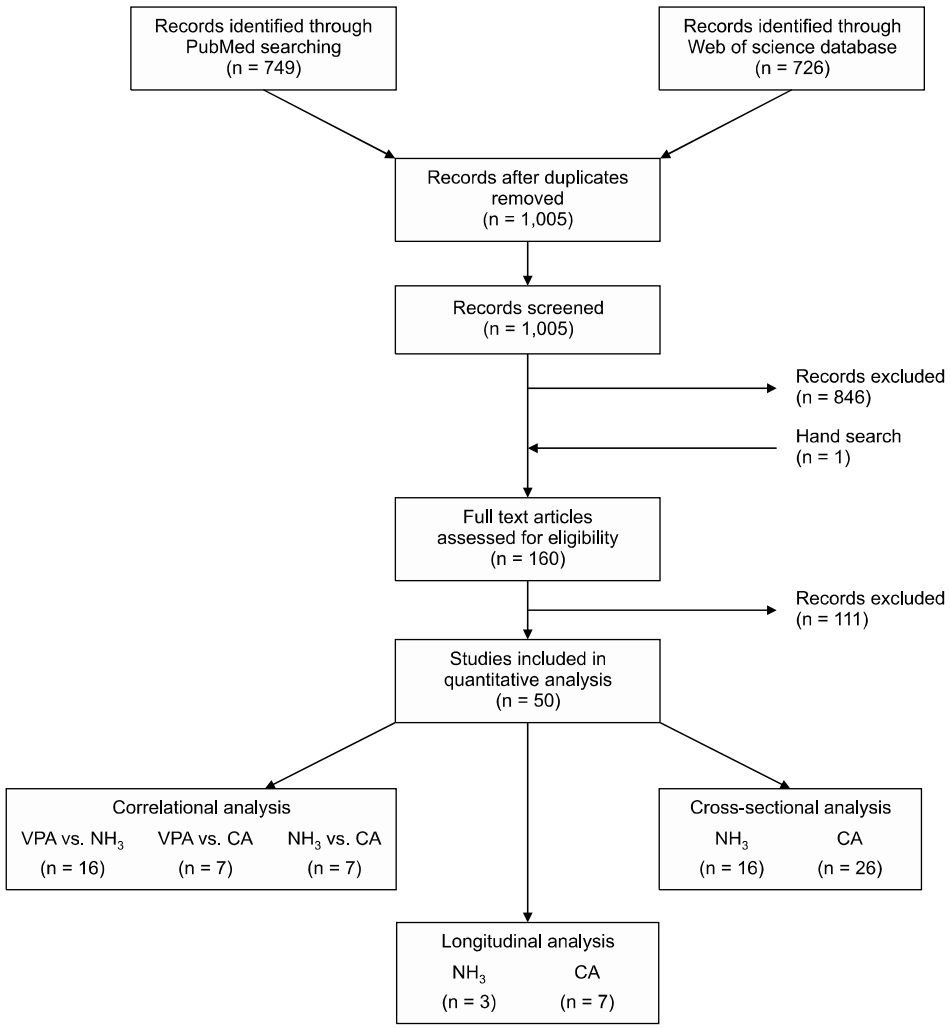

Fig. 1. A flow chart of the study selection process.
VPA, valproic acid; CA, carnitine; NH_3, ammonia.

DISCUSSION

To our knowledge, this is the first meta-analysis to assess the relationships between ammonia, free carnitine, and VPA. According to the pre- and post-VPA comparison and the cross-sectional comparison, VPA treatment significantly increased the blood ammonia level and decreased the blood carnitine level. The meta-correlational analysis revealed that the blood ammonia level had moderate associations with both VPA and free carnitine levels in blood. Furthermore, VPA level showed a weak correlation with free carnitine level in blood.

Hyperammonemia and hypocarnitinemia are well known as adverse metabolic effects of VPA treatment [2]. Ammonia is produced by the catabolism of proteins and other nitrogenated compounds. Under physiological conditions, ammonia exists as a constituent in body fluids and is transferred to the liver for its ultimate removal as urea. It is then excreted via the kidneys. Normally, circulating ammonia levels in blood are low, at less than 50 μmol/L (85 μg/dl) [46]. VPA is mainly metabolized by uridine diphosphate glucuronosyltransferases (UGTs) in the cytosol and partially via mitochondrial beta-oxidation and cytosolic omega-oxidation. The metabolites of VPA, such as valproyl-CoA, 2-propyl-4-pentenoate (4-ene VPA), and propionate, inhibit enzymes in the urea cycle, leading to an elevated blood ammonia level [50,57,58].

VPA treatment is also known as a cause of carnitine deficiency [2]. Carnitine, which is a carrier-type molecule required for the transport and oxidation of fatty acids in mitochondria, plays an important role in energy production [59]. Free plasma carnitine levels were significantly lower in patients who took VPA than in those who did not take VPA [24,26,36]. Although the mecha-

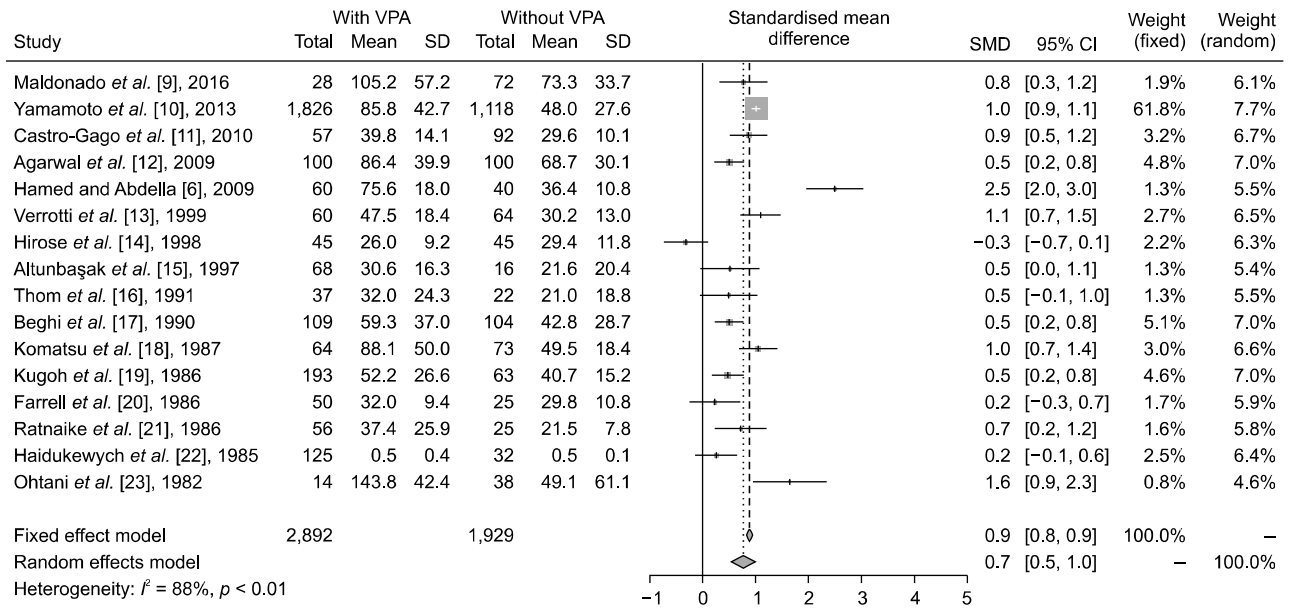

Fig. 2. Mean difference of blood ammonia levels between with and without valproic acid (VPA) treatment.
SD, standard deviation; CI, confidence interval; SMD, standardized mean difference.

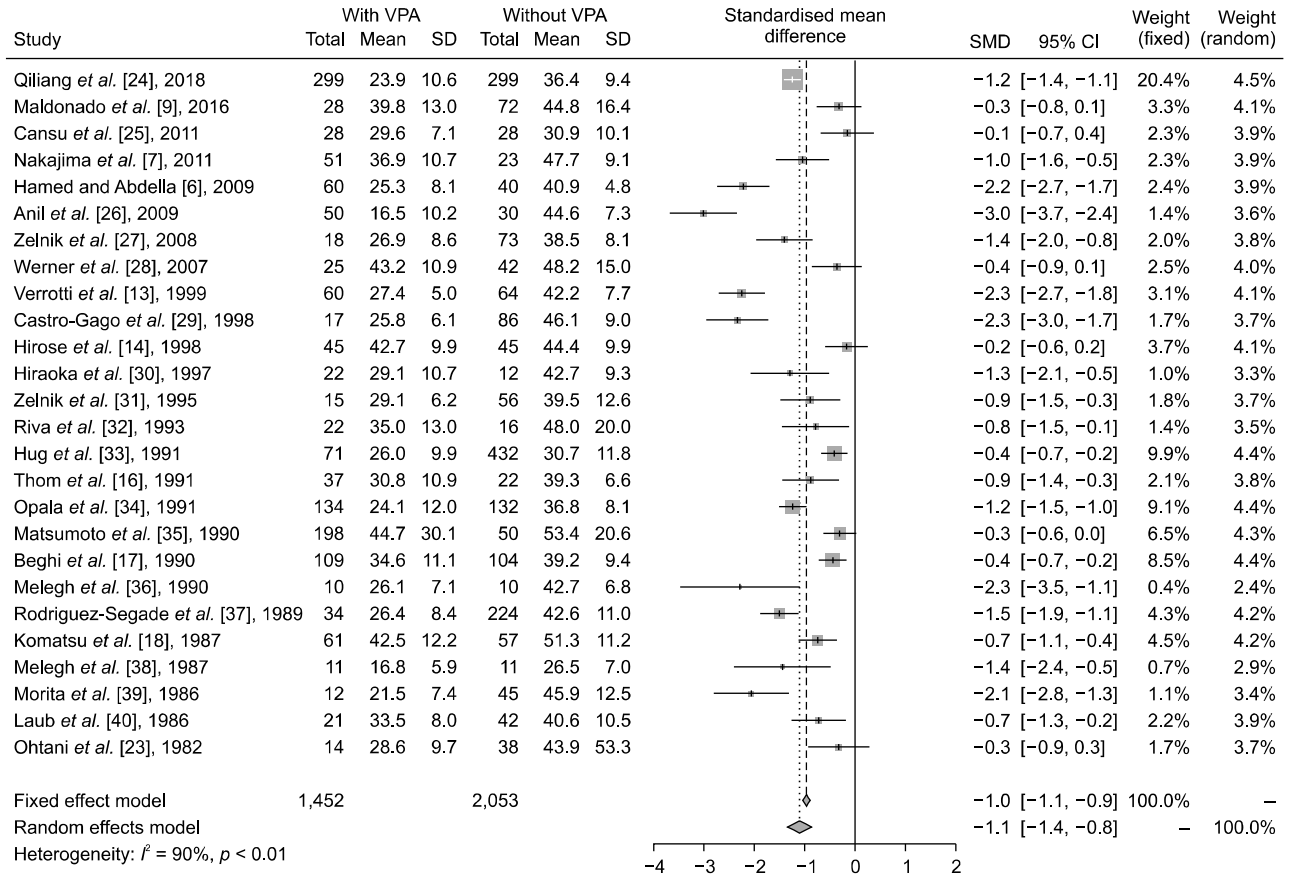

Fig. 3. Mean difference of blood free carnitine levels between with and without valproic acid (VPA) treatment.
SD, standard deviation; CI, confidence interval; SMD, standardized mean difference.

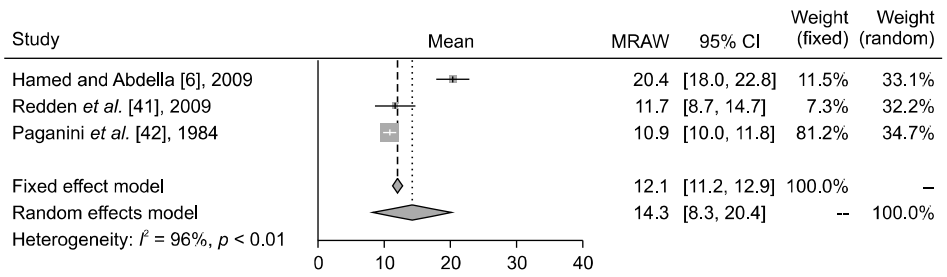

Fig. 4. Mean difference of blood ammonia levels after valproic acid treatment.
MRAW, raw mean; CI, confidence interval.

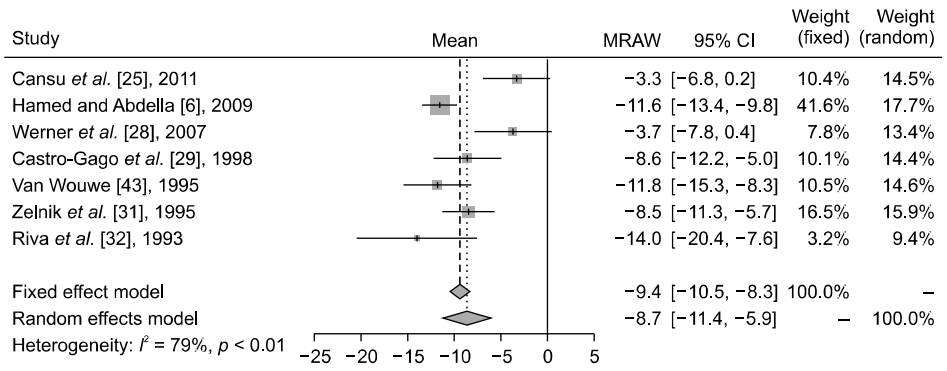

Fig. 5. Mean difference of blood free carnitine levels after valproic acid treatment.
MRAW, raw mean; CI, confidence interval.

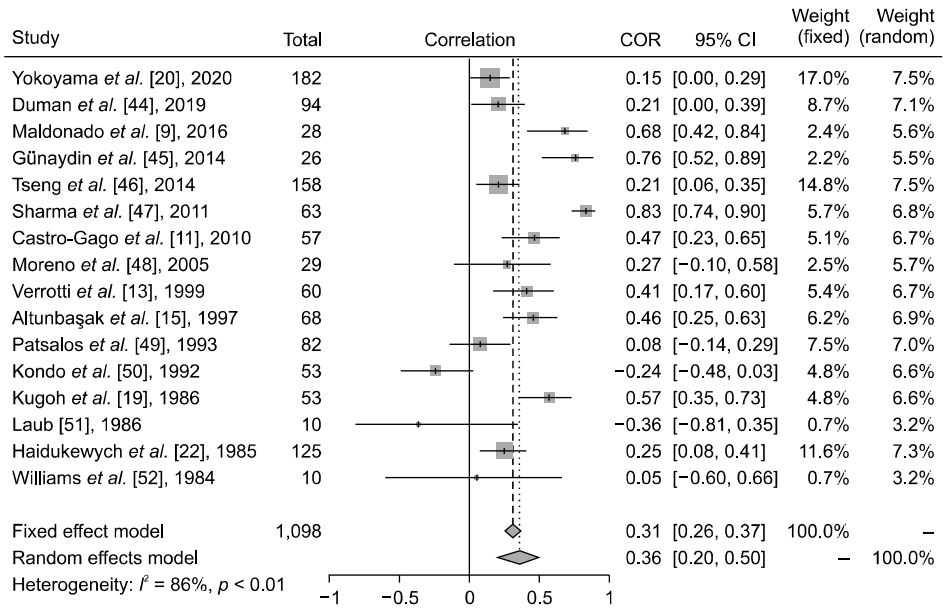

Fig. 6. Forest plot of standardized correlation coefficient between blood valproic acid and ammonia levels.
COR, correlation; CI, confidence interval.

nism of carnitine deficiency with VPA use is controversial, inhibition of carnitine biosynthesis via a decrease in alpha-ketoglutarate might be a potential cause [60].

Despite high heterogeneity, there are no studies in which the non-VPA group had a significantly higher ammonia level than the VPA group in a cross-sectional comparison, and all studies that included pre- and post-VPA comparisons showed a significantly elevated ammonia level after VPA treatment. Regarding free carnitine levels, there were no studies in which the non-VPA group had a significantly lower free carnitine level than the VPA group in a cross-sectional comparison, and most of the studies included in the pre- and post-VPA comparison showed a significant reduction in the free carnitine level after VPA treatment. Our results confirmed the abovementioned results in the meta-analysis of both the cross-sectional and

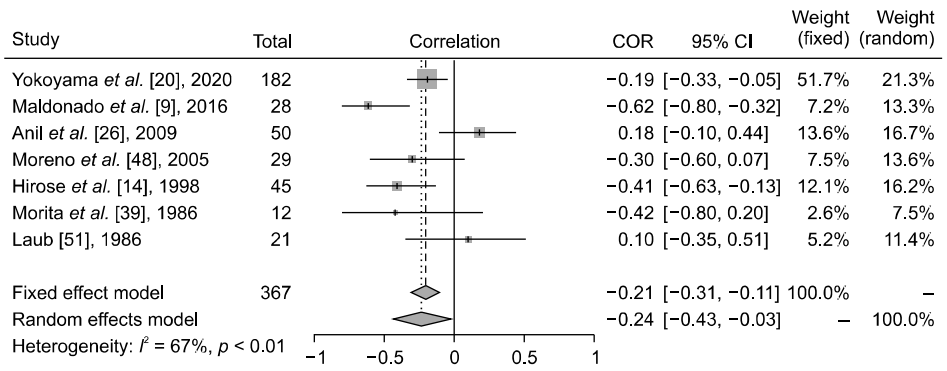

Fig. 7. Forest plot of standardized correlation coefficient between blood valproic acid and free carnitine levels.
COR, correlation; CI, confidence interval.

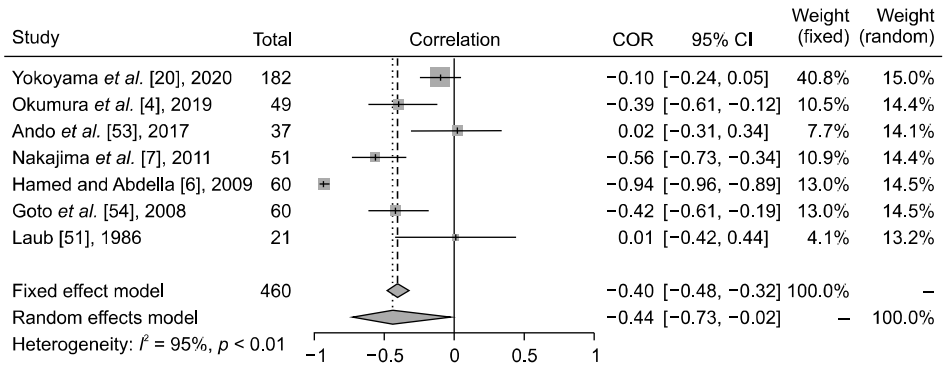

Fig. 8. Forest plot of standardized correlation coefficient between blood ammonia and free carnitine levels.
COR, correlation; CI, confidence interval.

pre-and post-VPA comparisons. Even though the mechanisms of hyperammonemia and hypocarnitinemia with VPA use are controversial, our pooled analysis robustly supports concern about these adverse metabolic effects in patients with long-term VPA use.

In the meta-correlational analysis, both ammonia and free carnitine levels in blood showed a significant association with blood VPA level. Although our results had significant heterogeneity, there were no studies showing a significantly negative correlation between VPA and ammonia and a significantly positive correlation between VPA and free carnitine. Blood level-dependent relationships might indicate dose-dependent relationships in clinical settings. Clinicians should be aware of hyperammonemia and hypocarnitinemia, especially in patients receiving high-dose VPA treatment.

Our results also demonstrated a significant correlation between ammonia and free carnitine levels in blood. Although carnitine deficiency can promote VPA-induced hyperammonemia via inhibition of the urea cycle [3,4], the clinical implications of our findings should be interpreted with caution due to the moderate effect size of the observed correlation. Patients with hyperammonemia do not necessarily have hypocarnitinemia. Carnitine is synthesized endogenously from two essential amino acids, lysine and methionine, and is also obtained primarily by the ingestion of meat and dairy products. Dietary intake of carnitine could affect blood levels, even after VPA treatment. Clinicians prescribing VPA should monitor both blood ammonia and free carnitine levels.

Our findings should be interpreted with caution due to several limitations of this meta-analysis. First, considerable heterogeneity, indicating variations in relationships among studies, may have affected our results, although we employed random effects models throughout the analyses to conservatively estimate the relationships. The effect size of the observed relationships should be interpreted with caution. Second, the analyses were based on a limited number of studies and subjects due to stringent inclusion/exclusion criteria. Nonetheless, the comprehensive search of two electronic databases may have limited the risk of reporting bias. Third, several potential confounding factors, such as age, reason for VPA treatment, dietary intake of carnitine, and use of other antiepileptics, were not included in our analyses. Indeed, it is important to note that meat and dairy products are sources of carnitine. Future studies assessing the effects of potential confounders on blood levels of ammonia and car-

nitine in patients treated with VPA are needed.

This was the first meta-analysis to assess the relationships between ammonia and free carnitine and VPA. In line with previous findings, VPA treatment was associated with both hyperammonemia and hypocarnitinemia in a blood level-dependent manner. Although the correlation between ammonia and free carnitine levels in blood was significant, the moderate strength of the correlation does not allow clinicians to infer free carnitine levels from the results of ammonia levels. Clinicians should measure both blood ammonia and free carnitine levels, especially in patients receiving high dosages of VPA.

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■ **Conflicts of Interest**

No potential conflict of interest relevant to this article was reported.

■ **Author Contributions**

Conceptualization: Saaya Yokoyama, Norio Yasui-Furukori. Methodology: Norio Sugawara. Data analysis; Kazushi Maruo. Overall study coordination; Norio Yasui-Furukori, Kazutaka Shimoda. Data interpretation: Saaya Yokoyama, Norio Sugawara, Kazushi Maruo, Norio Yasui-Furukori, Kazutaka Shimoda. Writing of the manuscript: Norio Sugawara, Kazushi Maruo, Saaya Yokoyama.

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