Klarica Domjanović et al. **Interaction between *ABCG2 421C>A* polymorphism and valproate in their effects on steady-state disposition of lamotrigine in adults with epilepsy**

Supplementary material

Table S1. Effects of variant alleles at genotyped UGT and ABC transporter loci on steady-state lamotrigine troughs or dose-adjusted troughs: main analysis, unadjusted models.

Table S2. Effects of variant alleles at genotyped UGT and ABC transporter loci on steady-state lamotrigine troughs or dose-adjusted troughs: main analysis, adjusted models.

Table S3. Effects of variant allele at *ABCG2 421C>A* on steady-state lamotrigine troughs or dose-adjusted troughs: supportive analysis with valproate co-treatment represented by valproate troughs.

Table S4. Effects of variant allele at *ABCG2 421C>A* on steady-state lamotrigine troughs or dose-adjusted troughs: supportive analysis separately in patients treated only with lamotrigine and patients treated with lamotrigine+valproate.

Figure S1. Effects of *CYP2C9* and *CYP2C19* polymorphysms on steady-state valproate troughs or dose-adjusted troughs.

Figure S2. Effects of variant alleles at genotyped UGT and ABC transporter loci on steady-state valproate troughs or dose-adjusted troughs.

Table S1. Effects of variant alleles at genotyped UGT and ABC transporter loci on steady-state lamotrigine troughs or dose-adjusted troughs: main analysis, unadjusted models. A separate model was fitted to log-transformed troughs for each polymorphism. The effects in each model are the same: treatment [lamotrigine (LAM) + valproate (VAL) or LAM], genotype (variant allele carriage or wild type homozygosity), treatment*genotype interaction and lamotrigine daily dose for measured troughs. Since four treatment-polymorphism interactions were tested, alpha level for the interaction term was set at 0.0125 to prevent false-positive findings. Effects are geometric means ratios (GMR) with 95% confidence intervals (CI). GMR for the interaction term is a ratio of two GMRs and informs about the relative difference in genotype or treatment (VAL) effects at different levels of treatment or genotype, respectively. A significant interaction term (GMR higher or lower than unity) indicates that the effect of variant allele on lamotrigine troughs is higher or lower, respectively, in VAL co-treated patients than in LAM only patients, or conversely, that the effect of VAL co-treatment is higher or lower, respectively, in variant allele carriers than in wild type homozygotes. Contrasts derived from the interaction term are without P-values, as they primarily serve to illustrate (a lack of) overlap of the effects in different subsets, not to test the significance of the effect within a subset.

Models by genotyped loci	Measured troughs		Dose-adjusted troughs	
	GMR (95% CI)	P	GMR (95% CI)	P
UGT1A4*3 142T>G	AIC=403.5		AIC=381.8	
Treatment (LAM + VAL vs. LAM)	2.60 (2.10-3.20)	<0.001	2.48 (2.02-3.04)	<0.001
Genotype (variant allele vs. wild type)	0.92 (0.75-1.13)	0.426	0.91 (0.74-1.12)	0.370
Treatment*genotype interaction	0.81 (0.53-1.22)	0.311	0.96 (0.64-1.45)	0.862
LAM + VAL vs. LAM at wild type	2.89 (2.35-3.55)		2.53 (2.07-3.09)	
LAM + VAL vs. LAM at variant allele	2.33 (1.62-3.35)		2.44 (1.71-3.47)	
Variant allele vs. wild type at LAM	1.02 (0.80-1.32)		0.93 (0.73-1.19)	
Variant allele vs. wild type at LAM+VAL	0.83 (0.59-1.15)		0.90 (0.65-1.24)	
LAM daily dose (by 25 mg)	1.16 (1.13-1.19)	<0.001	---	---
UGT2B7 -161C>T	AIC=404.7		AIC=382.6	
Treatment (LAM + VAL vs. LAM)	2.79 (2.27-3.42)	<0.001	2.53 (2.08-3.08)	<0.001
Genotype (variant allele vs. wild type)	1.00 (0.82-1.23)	0.979	1.00 (0.82-1.22)	0.986
Treatment*genotype interaction	0.93 (0.62-1.40)	0.730	0.95 (0.64-1.41)	0.794
LAM + VAL vs. LAM at wild type	2.89 (2.04-4.08)		2.60 (1.86-3.63)	
LAM + VAL vs. LAM at variant allele	2.69 (2.17-3.34)		2.47 (2.00-3.04)	
Variant allele vs. wild type at LAM	1.04 (0.79-1.36)		1.02 (0.79-1.33)	
Variant allele vs. wild type at LAM+VAL	0.97 (0.72-1.31)		0.97 (0.72-1.31)	
LAM daily dose (by 25 mg)	1.16 (1.13-1.19)	<0.001	---	---

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Table S1 continued.

Models by genotyped loci	Measured troughs		Dose-adjusted troughs	
	GMR (95% CI)	P	GMR (95% CI)	P
MDR1 exon 12 1236C>T	AIC=404.9		AIC=382.4	
Treatment (LAM + VAL vs. LAM)	2.70 (2.24-3.26)	<0.001	2.45 (2.04-2.94)	<0.001
Genotype (variant allele vs. wild type)	1.05 (0.87-1.26)	0.634	1.03 (0.86-1.23)	0.781
Treatment*genotype interaction	1.09 (0.75-1.59)	0.633	1.17 (0.81-1.68)	0.400
LAM + VAL vs. LAM at wild type	2.58 (1.91-3.49)		2.26 (1.69-3.03)	
LAM + VAL vs. LAM at variant allele	2.83 (2.26-3.54)		2.65 (2.13-3.29)	
Variant allele vs. wild type at LAM	1.00 (0.80-1.25)		0.95 (0.77-1.18)	
Variant allele vs. wild type at LAM+VAL	1.10 (0.81-1.48)		1.11 (0.83-1.49)	
LAM daily dose (by 25 mg)	1.16 (1.13-1.19)	<0.001	---	---
ABCG2 421C>A	AIC=394.0		AIC=375.2	
Treatment (LAM + VAL vs. LAM)	3.57 (2.80-4.54)	<0.001	3.10 (2.45-3.93)	<0.001
Genotype (variant allele vs. wild type)	1.13 (0.89-1.43)	0.325	1.13 (0.89-1.42)	0.323
Treatment*genotype interaction	2.19 (1.35-3.54)	0.001	1.89 (1.18-3.03)	0.009
LAM + VAL vs. LAM at wild type	2.41 (1.99-2.92)		2.26 (1.87-2.73)	
LAM + VAL vs. LAM at variant allele	5.28 (3.39-8.20)		4.26 (2.76-6.57)	
Variant allele vs. wild type at LAM	0.76 (0.59-0.98)		0.82 (0.64-1.05)	
Variant allele vs. wild type at LAM+VAL	1.67 (1.11-2.52)		1.55 (1.04-2.31)	
LAM daily dose (by 25 mg)	1.16 (1.13-1.19)	<0.001	---	---

Table S2. Effects of variant alleles at genotyped UGT and ABC transporter loci on steady-state lamotrigine troughs or dose-adjusted troughs: main analysis, adjusted models. Five models were fitted to log-transformed troughs. *Main effects model* with age, sex, body mass index, treatment [lamotrigine (LAM) + valproate (VAL) or LAM], genotypes at all four loci (variant allele carriage or wild type homozygosity) and lamotrigine daily dose for measured troughs. Four *interaction* models included the same main effects plus treatment*genotype interaction (a separate interaction model for each locus). Alpha level for the interaction term was set at 0.0125 to prevent false-positive findings. Effects are geometric means ratios (GMR) with 95% confidence intervals (CI). GMR for the interaction term is a ratio of two GMRs and informs about the relative difference in genotype or treatment (VAL) effects at different levels of treatment or genotype, respectively. A significant interaction term (GMR higher or lower than unity) indicates that the effect of variant allele on lamotrigine troughs is higher or lower, respectively, in VAL co-treated patients than in LAM only patients, or conversely, that the effect of VAL co-treatment is higher or lower, respectively, in variant allele carriers than in wild type homozygotes. In the case of a significant interaction term, variant allele effects are shown by treatment subset and treatment (VAL) effects are shown by genotype subset. However, they are given without P-values since primarily serve to illustrate (a lack of) overlap of the effects in different subsets, and not to test the significance of the effect within a subset.

Models by treatment*genotype interaction	Measured troughs		Dose-adjusted troughs	
	GMR (95% CI)	P	GMR (95% CI)	P
Main effects model (no interactions)	AIC=424.5		AIC=403.7	
Age (by 5 years)	0.99 (0.95-1.02)	0.409	0.99 (0.96-1.03)	0.782
Sex (men vs. women)	0.92 (0.76-1.11)	0.375	1.01 (0.84-1.22)	0.931
Body mass index (by 2 kg/m^2)	0.96 (0.92-1.03)	0.152	0.96 (0.91-1.00)	0.056
Treatment (LAM + VAL vs. LAM)	2.67 (2.22-3.22)	<0.001	2.42 (2.02-2.90)	<0.001
Genotypes				
*UGT1A4*3 142T>G* (variant allele vs. wild type)	0.92 (0.75-1.13)	0.429	0.89 (0.73-1.09)	0.254
UGT2B7 -161C>T (variant allele vs. wild type)	1.00 (0.82-1.23)	0.978	1.00 (0.82-1.22)	0.971
MDR1 exon 12 1236C>T (variant vs. wild type)	1.06 (0.89-1.27)	0.522	1.03 (0.86-1.23)	0.768
ABCG2 421C>A (variant allele vs. wild type)	0.95 (0.76-1.19)	0.678	0.98 (0.79-1.22)	0.862
LAM daily dose (by 25 mg)	1.16 (1.12-1.19)	<0.001	---	---

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Table S2 continued.

Models by treatment*genotype interaction	Measured troughs		Dose-adjusted troughs	
	GMR (95% CI)	P	GMR (95% CI)	P
+ treatment*UGT1A4*3 interaction	AIC=424.7		AIC=404.9	
Age (by 5 years)	0.99 (0.95-1.04)	0.425	0.99 (0.96-1.03)	0.789
Sex (men vs. women)	0.92 (0.76-1.12)	0.407	1.01 (0.84-1.22)	0.921
Body mass index (by 2 kg/m^2)	0.96 (0.92-1.01)	0.142	0.95 (0.91-1.00)	0.056
Treatment (LAM + VAL vs. LAM)	2.52 (2.03-3.13)	<0.001	2.39 (1.94-2.95)	<0.001
Genotypes				
*UGT1A4*3 142T>G* (variant allele vs. wild type)	0.89 (0.72-1.10)	0.294	0.88 (0.72-1.09)	0.247
UGT2B7 -161C>T (variant allele vs. wild type)	1.01 (0.83-1.24)	0.912	1.00 (0.82-1.22)	0.986
MDR1 exon 12 1236C>T (variant vs. wild type)	1.07 (0.89-1.28)	0.482	1.03 (0.86-1.23)	0.758
ABCG2 421C>A (variant allele vs. wild type)	0.94 (0.75-1.18)	0.618	0.98 (0.79-1.22)	0.848
Treatment**UGT1A4*3 142T>G* interaction	0.80 (0.52-1.21)	0.288	0.95 (0.63-1.43)	0.816
LAM daily dose (by 25 mg)	1.16 (1.11-1.20)	<0.001	---	---
+ treatment*UGT2B7 interaction	AIC=425.8		AIC=405.0	
Age (by 5 years)	0.99 (0.95-1.04)	0.413	0.99 (0.96-1.03)	0.787
Sex (men vs. women)	0.92 (0.76-1.11)	0.377	1.01 (0.84-1.22)	0.928
Body mass index (by 2 kg/m^2)	0.97 (0.92-1.02)	0.155	0.96 (0.91-1.00)	0.057
Treatment (LAM + VAL vs. LAM)	2.69 (2.18-3.31)	<0.001	2.43 (1.99-2.97)	<0.001
Genotypes				
*UGT1A4*3 142T>G* (variant allele vs. wild type)	0.92 (0.75-1.13)	0.436	0.89 (0.73-1.09)	0.259
UGT2B7 -161C>T (variant allele vs. wild type)	1.00 (0.82-1.23)	0.990	1.00 (0.81-1.22)	0.961
MDR1 exon 12 1236C>T (variant vs. wild type)	1.06 (0.88-1.27)	0.533	1.03 (0.86-1.23)	0.776
ABCG2 421C>A (variant allele vs. wild type)	0.95 (0.76-1.19)	0.682	0.98 (0.79-1.22)	0.865
Treatment**UGT2B7 -161C>T* interaction	0.98 (0.65-1.47)	0.909	0.98 (0.66-1.46)	0.922
LAM daily dose (by 25 mg)	1.16 (1.12-1.19)	<0.001	---	---
+ treatment*MDR1 interaction	AIC=425.8		AIC=404.3	
Age (by 5 years)	0.99 (0.95-1.02)	0.393	0.99 (0.96-1.03)	0.735
Sex (men vs. women)	0.92 (0.76-1.12)	0.409	1.02 (0.85-1.23)	0.847
Body mass index (by 2 kg/m^2)	0.97 (0.92-1.01)	0.154	0.96 (0.91-1.00)	0.056
Treatment (LAM + VAL vs. LAM)	2.64 (2.17-3.20)	<0.001	2.36 (1.96-2.85)	<0.001
Genotypes				
*UGT1A4*3 142T>G* (variant allele vs. wild type)	0.92 (0.76-1.12)	0.413	0.89 (0.73-1.08)	0.231
UGT2B7 -161C>T (variant allele vs. wild type)	1.01 (0.82-1.24)	0.935	1.01 (0.82-1.23)	0.942
MDR1 exon 12 1236C>T (variant vs. wild type)	1.08 (0.89-1.31)	0.451	1.06 (0.88-1.27)	0.565
ABCG2 421C>A (variant allele vs. wild type)	0.95 (0.76-1.19)	0.683	0.98 (0.79-1.22)	0.870
Treatment**MDR1 exon 12 1236C>T* interaction	1.10 (0.75-1.60)	0.639	1.19 (0.82-1.72)	0.355
LAM daily dose (by 25 mg)	1.16 (1.12-1.19)	<0.001	---	---

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Table S2 continued.

Models by treatment*genotype interaction	Measured troughs		Dose-adjusted troughs	
	GMR (95% CI)	P	GMR (95% CI)	P
+ treatment*ABCG2 interaction	AIC=414.7		AIC=396.9	
Age (by 5 years)	0.99 (0.95-1.02)	0.440	0.99 (0.96-1.03)	0.823
Sex (men vs. women)	0.94 (0.77-1.13)	0.488	1.03 (0.85-1.23)	0.789
Body mass index (by 2 kg/m^2)	0.96 (0.91-1.00)	0.075	0.95 (0.91-0.99)	0.028
Treatment (LAM + VAL vs. LAM)	3.49 (2.73-4.44)	<0.001	3.02 (2.38-3.83)	<0.001
Genotypes				
*UGT1A4*3 142T>G* (variant allele vs. wild type)	0.94 (0.79-1.14)	0.526	0.90 (0.74-1.10)	0.313
UGT2B7 -161C>T (variant allele vs. wild type)	0.97 (0.80-1.19)	0.800	0.97 (0.80-1.19)	0.786
MDR1 exon 12 1236C>T (variant vs. wild type)	1.06 (0.89-1.27)	0.494	1.03 (0.86-1.22)	0.748
ABCG2 421C>A (variant allele vs. wild type)	1.14 (0.89-1.45)	0.287	1.14 (0.90-1.45)	0.287
Treatment**ABCG2 421C>A* interaction	2.36 (1.39-3.64)	0.001	1.97 (1.22-3.18)	0.006
LAM+VAL vs. LAM at wild type	2.32 (1.89-2.83)		2.15 (1.77-2.62)	
LAM+VAL vs. LAM at variant allele	5.24 (3.38-8.15)		4.24 (2.75-6.54)	
Variant allele vs. wild type at LAM	0.76 (0.59-0.98)		0.81 (0.63-1.04)	
Variant allele vs. wild type at LAM+VAL	1.72 (1.14-2.62)		1.60 (1.07-2.62)	
LAM daily dose (by 25 mg)	1.16 (1.13-1.19)	<0.001	---	---

Table S3. Effects of variant allele at *ABCG2 421C>A* on steady-state lamotrigine troughs or dose-adjusted troughs: supportive analysis with valproate co-treatment represented by valproate troughs. Two general linear models were fitted to log-transformed lamotrigine troughs. Fully adjusted model with treatment***ABCG2* genotype interaction from Table S2 was refitted, however treatment was not considered as a binary variable rather, lamotrigine+valproate-treated patients were represented by their valproate troughs, while lamotrigine-only patients were considered to have zero values. Since valproate troughs were also log-transformed, the effect of increasing valoprate concentration on lamotrigine troughs is expressed by 2.71-fold increase. Parsimonious model was obtained by removing independent variables with P≥0.1. Effects are expressed as geometric means ratios (GMR) with 95% confidence intervals (CI). For the valproate trough***ABCG2* genotype interaction, GMR is a relative difference in the effect of a 2.71-fold increase in valproate trough between variant allele carriers and wild type homozygotes, i.e., a relative increase in the effect of the variant allele with a 2.71-fold increase in valproate concentration.

Models and effects	Measured troughs		Dose-adjusted troughs	
	GMR (95% CI)	P	GMR (95% CI)	P
Fully adjusted model	AIC=402.7		AIC=387.4	
Age (by 5 years)	0.98 (0.95-1.01)	0.197	0.99 (0.96-1.02)	0.513
Sex (men vs. women)	0.93 (0.77-1.12)	0.463	1.03 (0.86-1.23)	0.768
Body mass index (by 2 kg/m^2)	0.96 (0.92-1.01)	0.084	0.95 (0.91-0.99)	0.026
Valproate trough (by 2.71-fold)	1.24 (1.19-1.29)	<0.001	1.21 (1.17-1.26)	<0.001
*UGT1A4*3 142T>G* (variant allele vs. wild type)	0.93 (0.76-1.13)	0.464	0.90 (0.74-1.09)	0.260
UGT2B7 -161C>T (variant allele vs. wild type)	0.96 (0.79-1.17)	0.714	0.96 (0.79-1.17)	0.686
MDR1 exon 12 1236C>T (variant vs. wild type)	1.06 (0.90-1.26)	0.470	1.03 (0.87-1.22)	0.703
ABCG2 421C>A (variant allele vs. wild type)	0.99 (0.80-1.23)	0.919	1.01 (0.82-1.26)	0.898
Valproate trough**ABCG2 421C>A* interaction	1.13 (1.04-1.22)	0.003	1.11 (1.02-1.20)	0.011
Valproate trough at wild type	1.17 (1.13-1.21)		1.15 (1.12-1.19)	
Valproate trough at variant allele	1.32 (1.23-1.42)		1.26 (1.19-1.33)	
LAM daily dose (by 25 mg)	1.15 (1.12-1.18)	<0.001	---	---
Parsimonious model	AIC=384.7		AIC=368.0	
Body mass index (by 2 kg/m^2)	0.95 (0.91-0.99)	0.013	0.95 (0.91-0.99)	0.014
Valproate trough (by 2.71-fold)	1.24 (1.19-1.29)	<0.001	1.22 (1.17-1.26)	<0.001
ABCG2 421C>A (variant allele vs. wild type)	0.98 (0.78-1.20)	0.764	0.99 (0.81-1.27)	0.956
Valproate trough**ABCG2 421C>A* interaction	1.13 (1.05-1.22)	0.005	1.11 (1.02-1.20)	0.011
Valproate trough at wild type	1.17 (1.13-1.21)		1.16 (1.12-1.19)	
Valproate trough at variant allele	1.32 (1.23-1.42)		1.28 (1.19-1.37)	
LAM daily dose (by 25 mg)	1.15 (1.12-1.18)	<0.001	---	---

Table S4. Effects of variant allele at *ABCG2 421C>A* on steady-state lamotrigine troughs or dose-adjusted troughs: supportive analysis separately in patients treated only with lamotrigine and patients treated with lamotrigine+valproate. Fully adjusted main effects general linear models were fitted to log-transformed troughs in the two patient subsets. Effects are geometric means ratios (GMR) with 95% confidence intervals (CI).

	Measured troughs		Dose-adjusted troughs	
Patient subset and model effects	GMR (95% CI)	P	GMR (95% CI)	P
Lamotrigine-only patients (n=131)				
Age (by 5 years)	0.98 (0.94-1.03)	0.502	1.00 (0.96-1.05)	0.870
Sex (men vs. women)	0.97 (0.74-1.27)	0.801	1.23 (0.95-1.59)	0.118
Body mass index (by 2 kg/m^2)	0.94 (0.88-1.00)	0.058	0.91 (0.86-0.97)	0.005
*UGT1A4*3 142T>G* (variant allele vs. wild type)	1.03 (0.79-1.34)	0.824	0.92 (0.71-1.19)	0.522
UGT2B7 -161C>T (variant allele vs. wild type)	1.05 (0.79-1.39)	0.722	1.02 (0.78-1.35)	0.864
MDR1 exon 12 1236C>T (variant vs. wild type)	1.02 (0.81-1.28)	0.872	0.96 (0.76-1.21)	0.717
ABCG2 421C>A (variant allele vs. wild type)	0.75 (0.57-0.98)	0.036	0.79 (0.60-1.03)	0.082
Lamotrigine daily dose (by 25 mg)	1.13 (1.09-1.17)	<0.001	---	
Lamotrigine+valproate patients (n=74)				
Age (by 5 years)	0.97 (0.94-1.03)	0.453	0.97 (0.93-1.02)	0.251
Sex (men vs. women)	0.88 (0.68-1.14)	0.338	0.83 (0.65-1.07)	0.142
Body mass index (by 2 kg/m^2)	0.99 (0.92-1.06)	0.726	1.01 (0.94-1.07)	0.851
*UGT1A4*3 142T>G* (variant allele vs. wild type)	0.80 (0.60-1.07)	0.129	0.90 (0.68-1.19)	0.450
UGT2B7 -161C>T (variant allele vs. wild type)	0.95 (0.72-1.25)	0.702	0.95 (0.73-1.24)	0.701
MDR1 exon 12 1236C>T (variant vs. wild type)	1.18 (0.90-1.56)	0.233	1.14 (0.87-1.48)	0.339
ABCG2 421C>A (variant allele vs. wild type)	1.64 (1.14-2.36)	0.009	1.56 (1.10-2.22)	0.014
Lamotrigine daily dose (by 25 mg)	1.21 (1.16-1.27)	<0.001	---	

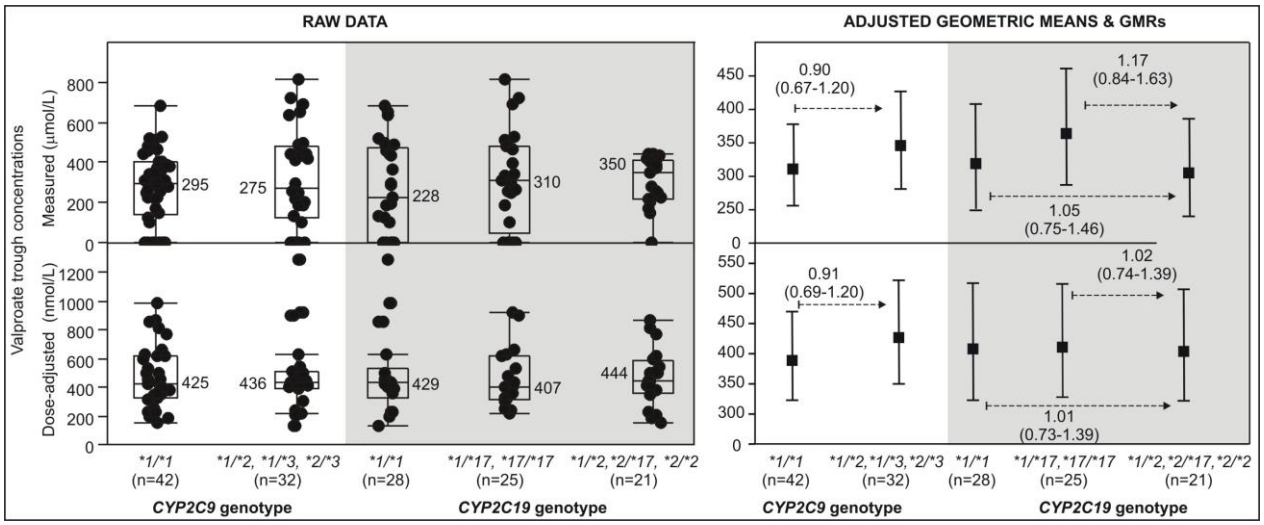

Figure S1. Effects of *CYP2C9* and *CYP2C19* polymorphisms on steady-state valproate troughs or dose-adjusted troughs. Measured (upper row) and dose-adjusted (lower row) steady-state morning valproate trough concentrations in lamotrigine+valproate-treated patients by *CYP2C9* and *CYP2C19* genotype. Raw data (left) are shown as individual values (circles), medians (horizontal lines, numerical values), quartiles (boxes) and inner fences (bars). Points outside fences are outliers. Adjusted geometric means and geometric means ratios (GMRs) (right) with 95% confidence intervals were obtained from a general linear model fitted to log-transformed valproate troughs (or dose-adjusted troughs) with the following effects: *CYP2C9* genotype *(*1/*1* or **2* or **3* allele carriage), *CYP2C19* genotype (**1/*1*, combined **1/*17* and **17/*17*, or **2* allele carriage), age, sex, body mass index and valproate dose (for measured troughs). In the analysis of measured troughs, by 300 mg higher valproate dose was associated with 20% (95%CI 11-28) higher valproate troughs. In both analyses, valproate troughs were higher in women than in men (measured, with dose as a covariate, by 15% [95%CI 2-29], dose-adjusted by 16% [95%CI 3-30]). There were no effects of age or body mass index (all GMRs closely around unity).

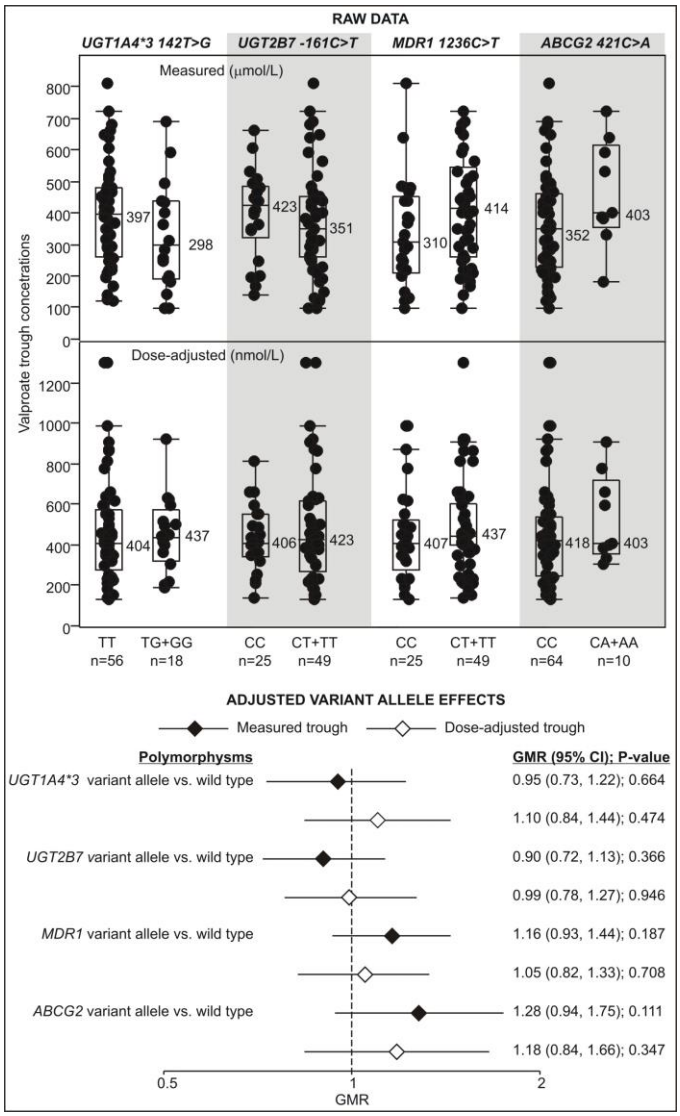

Figure S2. Effects of variant alleles at genotyped UGT and ABC transporter loci on steady-state valproate troughs or dose-adjusted troughs. Upper panel: raw measured and dose-adjusted steady-state morning valproate troughs in lamotrigine+valproate-treated patients by genotype (wild type homozygosity or variant allele carriage) at the UGT and transporter loci. Shown are individual values (circles), medians (horizontal lines, numerical values), quartiles (boxes) and inner fences (bars). Points outside fences are outliers. Lower panel: adjusted effects (geometric means ratios, GMRs) of variant allele carriage by genotyped locus (a separate model was fitted to log-transformed measured or dose-adjusted troughs with the following effects: variant allele carriage vs. wild type, age, sex, body mass index and valproate dose for measured troughs). Effects of valproate dose and sex were virtually identical in all current models as in the analysis depicted in Figure S1, while age and body mass index consistently had no effect.