Supplemental Information: Online Resource 1

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Bearing variant alleles at polymorphic uridine glucuronosyltransferase loci *UGT2B7 -161C>T* (rs7668258) or *UGT1A4*3 c.142T>G* (rs2011425) has no relevant consequences for lamotrigine troughs in adults with epilepsy

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Methods to achieve conditional exchangeability

We adopted the concept of causality as set forth by Pearl [1-4] and as implemented in R package *daggity* [5] – we generated a directed acyclic graph (DAG) in order to adequately identify roles of individual variables, define those that need to be controlled for in order to "exclude" and not introduce confounding/bias (as much as reasonably possible) (i.e., to "close" and not to "open" "backdoor paths" between the presumed cause and the outcome of interest) [6], and to identify sources of confounding that are practically almost impossible to control (considering, for example, a large number of potentially relevant polymorphisms). The essential elements of the emulated trials conceived to evaluate the effects of interest are shown in Figure S1 and most of the substantiative knowledge needed to "build" the graph is elaborated in the main text (additional elaboration added where appropriate): 1. The (potential) causal path (i.e., the one investigated) starts with a black circle (Fig S1) indicating genotype at the evaluated polymorphism – *UGT2B7 -161C>T* [CC (wild-type), CT or TT] or *UGT1A4*3 c.142T>G* [TT(wild-type) or TG/GG taken together since <1% patients were variant homozygous] – with a thick full black arrow projecting to the respective (UGT2B7 or UGT1A4) enzyme activity (depicted as a dark gray-black outlined circle), and a further thick black arrow projecting to the outcome(lamotrigine trough) depicted by a black circle. Typically, such a path indicates a causal effect of the "starting point" (exposure, treatment) on the outcome. The setting is specific in that the actual "exposure" or "treatment" is UGT2B7 or UGT1A4 enzyme activity important for lamotrigine clearance and known, at least *in vitro*, to be affected by the respective polymorphisms. However, as it cannot be measured directly *in vivo*, it remains unmeasured (hence – gray). The starting point, i.e., the genotype, on the other hand, can be determined and is used as a "proxy" of exposure. It has no other ways of affecting the outcome but by the effect on enzyme activity, has no effect on any other variable in the constellation of various elements, and thus qualifies as an instrumental variable. Still, it is not an "ideal" instrument. Since the two polymorphisms are each in complete linkage equilibrium (LD) with a variety of other polymorphisms in the respective genes, by identifying a specific genotype at these polymorphisms, one identifies subjects with particular broader genetic makeups that include all the linked polymorphisms (which, however, remain undetermined). Another shortcoming of this instrument is that it is not "exhaustive" – both *UGT2B7* and *UGT1A4* genes each harbor several tens of polymorphisms that are not linked (or are not known to be linked) to the genotyped loci and that might, although this is currently not known for a fact, also reflect on the respective enzyme activity: hence, they are depicted as "competing instrument" which, however, remains unmeasured (depicted as a dark gray circle); 2. Other elements in the DAG are depicted by blue circles indicating outcome ancestors (may affect the outcome) and their parents, or by red circles indicating "classical confounders" (may affect both the outcome and the exposure) and their parents; 3. Full arrows in the DAG indicate causality: thick black arrows indicate the assessed causal path, while thinner gray arrows depict the effect of parent variables on their descendants, i.e., outcome ancestors or "classical confounders". Black dashed arrows indicate "biasing paths" (with the respect to the investigated causal path), i.e., (known or very likely) effects of outcome ancestors on the outcome, and the effects of "classical confounders" on both the outcome and the exposure (UGT2B7 or UGT1A4 enzyme activity). These biasing paths might be direct, or mediated through one or more "downstream" variables (mediators);

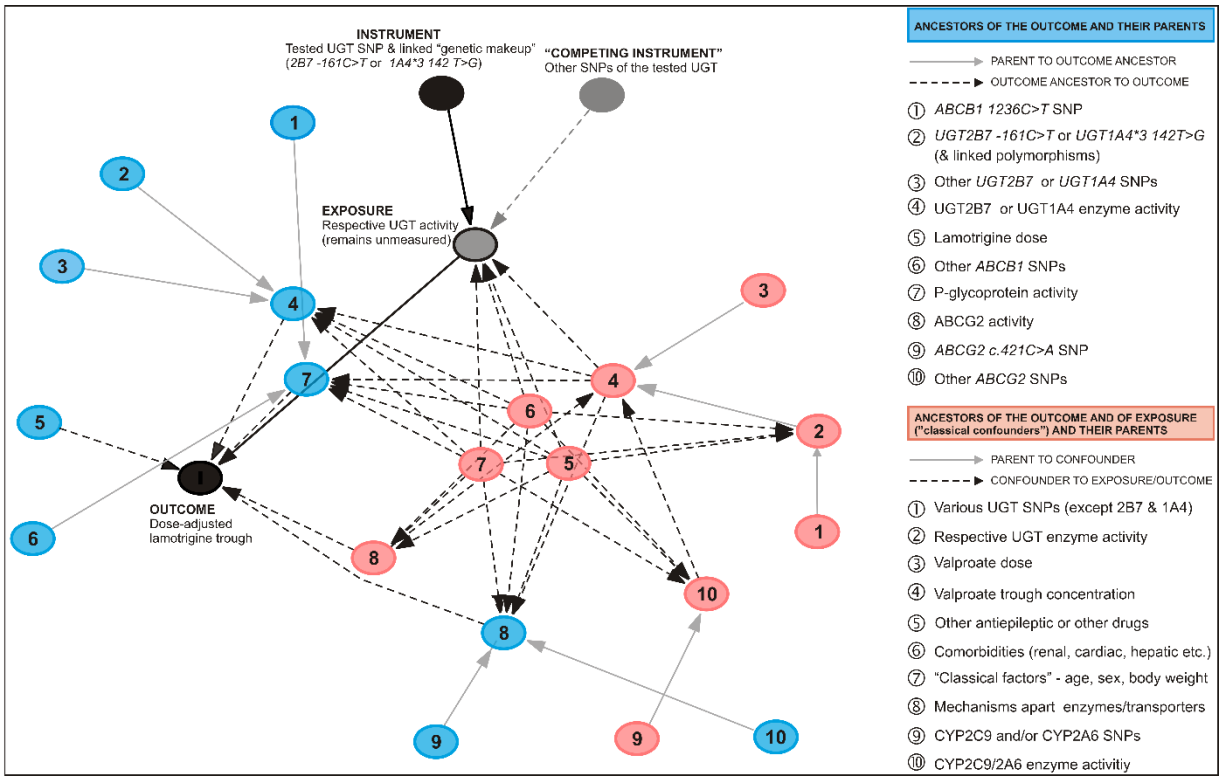

Fig S1 Directed acyclic graph (DAG, generated using package *daggity*) representing variables of interest in the investigated setting, and their roles. Black & dark gray-black outlined circles connected with thick black arrows depict the relationship of interest, i.e., the presumed (potential) causal path that is investigated: the outcome of interest is dose-adjusted lamotrigine trough; the actual exposure ("treatment") is the activity of the UGT2B7 or UGT1A4 enzyme(s) which, however, remains unmeasured but it is "represented" by an instrument: genotype at the respective polymorphism [*UGT2B7 -161C>T* (CC, CT or TT); or *UGT1A4*3 c.142T>G* (TT or TT/TG taken jointly)] and polymorphisms linked to it, which also are not measured (but are acknowledged based on the existing knowledge). The concept implies that the detected genotype represents a broader genetic makeup (includes polymorphisms in LD) that differs in wild-type (wt), heterozygous, and variant homozygous subjects with consequently different enzyme activity (i.e., results in "treated" vs. "control" activity). A completely dark gray circle denoted as a "competing instrument" indicates that the used instrument is not "ideal": it is known that both *UGT2B7* and *UGT1A4* genes harbor many other polymorphisms which are not (or are not known to be) linked with the typed polymorphisms and these (although, this is also not known for a fact, but is assumed) might also reflect on the respective enzyme activity ("exposure"). All black dashed arrows depict "direct" or "mediated" effects on only the outcome (arising from outcome ancestors – blue circles) or on both the outcome and the "exposure" (arising from confounders – red circles) that bias the investigated causal path. Most or some of the outcome ancestors and confounders have "parents", i.e., variables by which they are defined (or given birth) – causal effects of parents on their "children" (descendants) are denoted as gray full arrows. Note: in some cases, confounders affect the outcome through their "downstream" effects on the outcome ancestors (i.e., "red circles work 'through' the effects on blue circles").

4. It should be noted that the lack of arrows emerging from the outcome and ending in "exposure" or in any outcome ancestor or confounder (or their parent) illustrates that the condition of *no reverse causation* is met: (i) lamotrigine does not affect genotype (*UGT* or *ABCB1* and *ABCG2* genes); (ii) since blood samples were taken after at least 21 days of treatment, the initial induction of UGT1A4 and UGT2B7 enzymes (or other UGT enzymes) by lamotrigine has been completed, hence UGT1A4 or UGT2B7 activity (whether considered as "exposure" or as "outcome ancestor") or activity of any other UGT enzyme is not affected by lamotrigine; (iii) consequently, valproate levels (a confounder) are not affected by lamotrigine; (iv) lamotrigine does not affect the activity of ABCB1 or ABCG2 transporters; and (v) clearly, lamotrigine has no "reverse" effect on classical confounders/outcome ancestors like age, body weight, comorbidities or comedication. 5. Blue circles in Fig S1 – outcome ancestors: (i) we considered lamotrigine dose as a particularly relevant outcome ancestor; (ii) depending on which one was considered "exposure", UGT2B7 or UGT1A4 enzyme activity was considered an outcome ancestor, with several obvious and potential "parents" – polymorphisms *UGT2B7 -161C>T* or *UGT1A4*3 c.142T>G* (depending on which one was considered "instrument") and "other" (undetermined) polymorphism in the respective genes (depending on which were considered "competing instrument"), and antiepileptic (AED) or other drugs, comorbidities, exposure to valproate and "classical" factors such as age, sex, body weight – that might affect the enzyme activity (and are, otherwise, considered as confounders – see below); (iii) activity of the ABCG2 transporter (to which lamotrigine is a substrate) with several obvious and potential parents – polymorphism *ABCG2 c.421C>A*, other *ABCG2* polymorphisms, exposure to valproate (although valproate is not an ABCG2 substrate [7], there is evidence from human placental tissue that valproate might alter expression of the ABCG2 protein [8]), various AEDs and non-AED drugs that might reflect on ABCG2 activity, and comorbidities and classical factors such as age, body weight and sex, that might potentially reflect on ABCG2 activity (and are, otherwise, considered as confounders – see below); (iv) activity of the ABCB1 transporter (P-glycoprotein), with several obvious and potential parents – polymorphism *ABCB1 1236C>T* and other *ABCB1* polymorphisms, and other factors (as in the case of ABCG2 or UGT activity) that are otherwise considered as confounders (as in the case of ABCG2, valproate is not an ABCB1 substrate, but might alter expression of ABCB1 [7, 8]). 6. Red circles in Fig S2 – ("classical") confounders: (i) exposure to valproate. It is well known that valproate inhibits lamotrigine-metabolizing UGTs, i.e., UGT1A4 and UGT2B7, hence, depending on which is considered "exposure" and which is considered "outcome ancestor" valproate affects both the exposure and the outcome. It can further affect outcomes by affecting ABCB1 and/or ABCG2 activity (as mentioned). There are many obvious and potential "parents" to exposure to valproate: valproate dose; polymorphisms in genes encoding other UGTs (apart form UGT1A4 and UGT2B7) that affect activity of the respective enzymes involved in valproate clearance [9]; closely related "parents" are drugs and comorbidities that might reflect on activity of these UGTs (directly or through gene expression); activity of CYP2C9 and 2A6 (key for valproate clearance [10]) affected by polymorphisms and/or by other AED and non-AED drugs and/or comorbidities or "general" subject characteristics as age, sex, body weight and including unknown (assumed) mechanisms apart from the effects on enzymes and transporters; (ii) other AEDs or non-AED drugs, that might affect "exposure" (UGT2B7 or UGT1A4 activity) directly or by affecting exposure to valproate by affecting relevant UGT and/or CYP activity, or via any other mechanism apart from enzymes and transporters, and may affect the

outcome via effects on any of the outcome ancestors (UGT1A4 or UGT2B7 activity, ABCB1 activity, ABCG2 activity, or via a further mediator, which is exposure to valproate); (iii) comorbidities and/or "classical" factors like age, body weight and sex – that might affect both the "exposure" and the outcome via the same possible routes as depicted for comedication (apart from valproate).

Figure S2 shows the same DAG as Figure S1, but with different messages summarizing the results of the attempts to control confounding and, potentially, specifically estimate the tested causal path denoted by the black circles (instrument and outcome) connected by thick black arrows "via" exposure (dark gray-black outlined circle). All white circles indicate (potential) sources of confounding bias that were controlled for by different means. *Daggity* enables one to identify the "minimum adjustment set", i.e., the smallest possible set of confounders that need to be conditioned upon to close all backdoor paths, i.e., prevent spurious associations between the instrument ("exposure") and the outcome – i.e., it may not be needed to condition upon all of them individually to achieve the goal: for example, valproate dose is a direct and the most important parent to valproate trough – an important confounder – but, if one conditions on valproate trough, one does not need to condition on its parent valproate dose. *Daggity* "recognizes" confounders (and their parents or descendants) by their "classical" definition which includes links to both exposure and the outcome. It does not "recognize" variables that affect only the outcome (i.e., outcome ancestors and their parents) – but these also need to be controlled to evaluate the causal path of interest [6]. In Figure S2, white black-outlined circles depict confounders/outcome ancestors that we directly conditioned upon by different means: i) inclusion/exclusions criteria – relevant comorbidity and comedication (AED or non-AED) apart from valproate; ii) definition of the outcome as "dose-adjusted" lamotrigine trough controls for the effect of the outcome ancestor "lamotrigine dose"; iii) statistical adjustment (entropy balancing) – *ABCB1 1236C>T* polymorphism (and polymorphisms that are in a strong or complete LD), thus "removing" their contribution to P-glycoprotein activity; *UGT2B7 -161C>T* or *UGT1A4*3 c.142T>G* polymorphism when considered as a covariate (and polymorphisms that are in a strong on complete LD with it), thus "removing" their impact on their descendant "enzyme activity"; *ABCG2 c.421C>A* polymorphism (and polymorphisms in a strong on complete LD), thus removing its contribution to ABCG2 activity; valproate trough concentrations and classical factors age, body weight and sex, thus removing their impact (direct or mediated) on exposure and the outcome. White-red outlined circles in Figure 2S depict confounder parents that were controlled "indirectly", i.e., by directly controlling their children: by conditioning on valproate trough, blocked is the influence of all of its ancestors, i.e., valproate dose, CYP2C9/2A6 polymorphisms reflecting on enzyme activity, polymorphisms/activity of UGTs (other than 2B7 and 1A4) and others that are known to- or might affect valproate clearance. Light gray circles in Figure S2 denote elements that were not controlled for and that remain potential sources of confounding bias: i) "competing instrument"– an (apparent) effect of the assessed instrument on the outcome, or a lack of an effect might be due to the biasing effect of the "competing instrument" on exposure; ii) *UGT2B7* or *UGT1A4* polymorphisms other than the genotyped ones (and their linked polymorphisms) when considered as outcome ancestors might have affected the activity of the respective enzyme; similarly, *ABCG2* and *ABCB1* polymorphisms other than genotyped ones (and their linked polymorphisms) might have affected the activity of the respective transporters. Note, however, that

UGT2B7/UGT1A4 activity, P-glycoprotein activity and ABCG2 activity are denoted as light gray-black outlined circles: their activity was partially "controlled for" by controlling other factors, but not in full since their mentioned parents were not controlled for. Finally, there is always a possibility of some unmeasured confounding arising from factors that have not been known so far to impact lamotrigine concentrations. Open biasing paths from "competing instrument" to exposure, and those coming into only "partly controlled" outcome ancestors from their parents are depicted as full gray arrows. The dashed black arrows indicate biasing paths emerging from "only partly controlled" outcome ancestors towards the outcome. Other black dashed lines are blocked and indicate blocked backdoor paths.

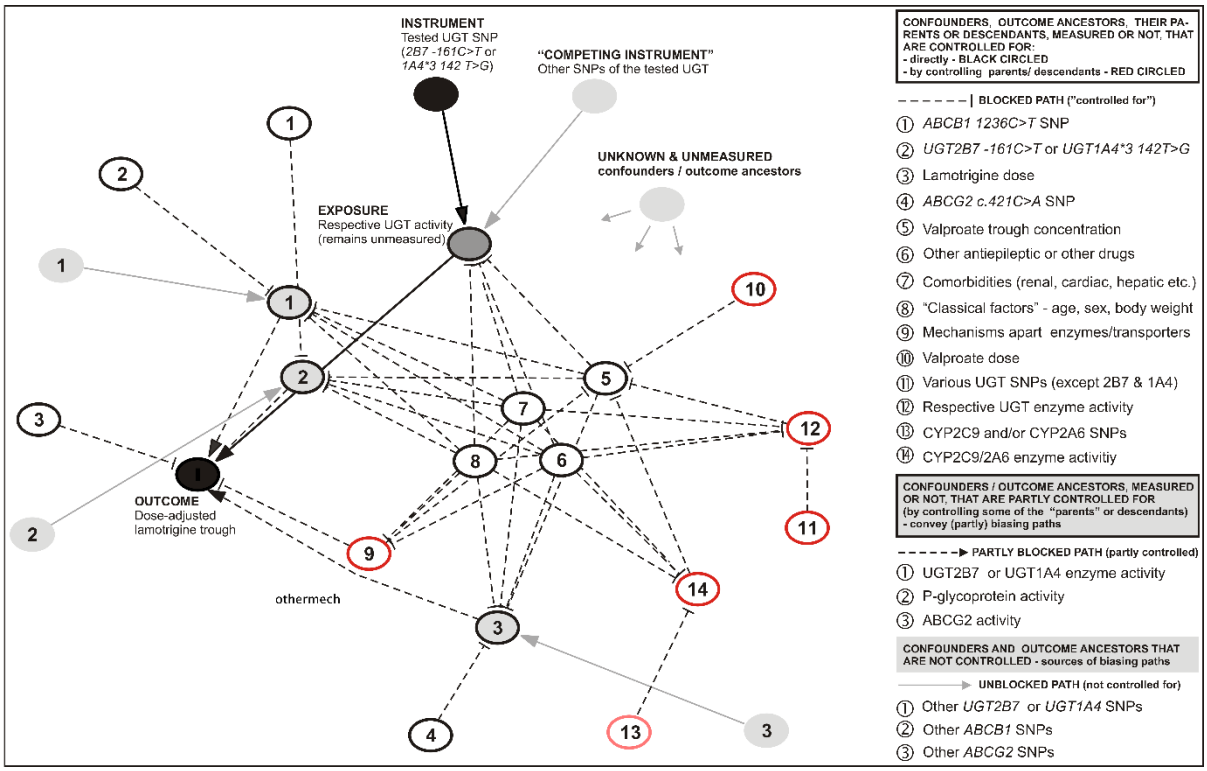

Figure S2. DAG from Figure S1 with denoted ancestors/confounders and their parents and/or descendants that were fully controlled for (white circles – black outline if controlled directly, red outline if controlled by controlling descendants), that were partly controlled for (light gray-black outlined circles), or were not controlled for (light gray circles). The full gray arrow from "competing instrument" to "exposure" indicates its (presumed) effect on the exposure. Other full gray arrows indicate effects "coming in" to partly controlled outcome ancestors from (some of) their parents. Dashed black arrows indicate biasing effects of outcome ancestors on the outcome. Other, blocked, dashed lines indicate blocked backdoor paths.

Therefore, the whole concept *a priori* acknowledged existence of otherwise known or reasonably suspected confounders/outcome ancestors that remained uncontrolled– this emphasized the need to evaluate t susceptibility of the generated estimates to unmeasured confounding.

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Sensitivity of GMR to unmeasured confounding

The method used to address sensitivity of the observed estimates to unmeasured confounding, i.e., to generate bias-corrected estimates was developed for relative risks (RR), while geometric means ratio (GMR) was the outcome measure used in the present study. Still, we deemed the method appropriate for the purpose due to certain common features of RR and GMR: (i) both are exponents of difference in means of ln-transformed quantities (risk or a right-tailed continuous variable like drug concentration); (ii) both ln(risk) and ln(right-tailed continuous variable) have a normal distribution and their interpretation is similar as they provide information about a relative difference between treatment and control. If for a treatment vs. control RR >1.0, e.g., 1.5, it means relatively by 50% higher risk with treatment, just as is the case with GMR: if 1.5, it means relatively by 50% higher value of the measured quantity with treatment.

Weights assigned to "treated" and "control" subjects

The entropy balancing algorithm defines constraints on the moments of covariates in a balancing set and then searches for weights to be assigned to each "treated" and "control" subject (or subjects in other subsets if more than two) so that the defined constraint is met [1]. For example, if a "balancing set"

contains several continuous and several categorical covariates, the procedure searches for weights that will, in the end, result in identical means and proportions across the balanced subsets (i.e., standardized mean differences will be 0), or in closely similar means/proportions so that the standardized mean differences will be <0.1 (a limit typically used to identify adequate balance). At the same time, the mean of the weights in each subject subset will be 1.0 with a moderate standard deviation, weight ranges will overlap and there will be no extreme weights that would require trimming [to avoid overt dependence on a few highly (under)weighted individuals]. For this to be possible, raw data across the subject subsets should show a reasonable level of overlap in the values of all covariates. When such an overlap is modest, some assigned weights will be rather high (or, reciprocally, low), standardized differences will differ from 0 or might exceed the limit of 0.1. However, there are also situations where raw data overlap is so poor that entropy balancing is not possible. In the present study, for all "emulated trials", entropy balancing was successful with all standardized differences being 0 and with assigned weights within reasonably narrow ranges (Table S1).

Table S1 Summary of weights assigned by entropy balancing in all "emulated trials" in the present study.

	Mean	Min - Max	RSD
Overall *UGT2B7 -161 C>T*			
CC	1.00	0.496-2.243	0.327
CT	1.00	0.673-1.427	0.161
TT	1.00	0.565-2.256	0.331
Overall *UGT1A4 c.142T>G*			
TT	1.00	0.753-1.455	0.118
TG/GG	1.00	0.311-2.463	0.432
UGT2B7 -161C>T at valproate 0/BLOQ			
CC	1.00	0.508-1.927	0.265
CT/TT	1.00	0.827-1.120	0.066
UGT2B7 -161C>T at valproate >0/BLOQ			
CC	1.00	0.539-2.391	0.453
CT/TT	1.00	0.598-1.386	0.181
UGT1A4 c.142T>G at valproate 0/BLOQ			
TT	1.00	0.761-1.320	0.106
TG/GG	1.00	0.485-3.180	0.377
UGT1A4 c.142T>G at valproate >0/BLOQ			
TT	1.00	0.713-1.295	0.115
TG/GG	1.00	0.452-2.498	0.514

References

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