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FULL-LENGTH ORIGINAL RESEARCH

Genetic variation in dopaminergic activity is associated with the risk for psychiatric side effects of levetiracetam

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Summary

Purpose: **Levetiracetam (LEV) is a highly effective antiepileptic agent. A clinically relevant psychiatric complication of LEV treatment, however, is the provocation of irritability and aggression. Recent behavioral research indicates that personality traits may predispose to these side effects. To assess the genetic basis of the adverse psychotropic profile of LEV, a candidate gene-based two-stage association study was conducted.**

Methods: **Polymorphisms were a priori selected according to their relevance for impulsivity and reactive-impulsive aggression. Based on data from both stages, a Bonferroni-corrected joint meta-analysis was computed.**

Key Findings: **Stage I analysis included 290 patients with epilepsy and revealed a higher load of adverse psychotropic side effects of LEV in patients carrying genetic variants associated with decreased dopaminergic activity: rs1611115 (dopamine-β-hydroxylase, DBH), rs4680 (catechol-O-methyltransferase, COMT), and rs1800497 (dopamine receptor D2-associated ANKK1 TAQ-1A). Stage II analysis including 100 patients with epilepsy, and joint meta-analysis confirmed the effect of the rs1800497 polymorphism (Bonferroni corrected significance of the joint meta-analysis, p = 0.0096).**

Significance: **Confirming the suggestion from behavioral observations that patients might be predisposed to develop irritation and aggression under treatment with LEV, the findings provide first evidence of an association of genetic variation in dopaminergic activity and the risk for psychiatric complications of LEV treatment. Replication and further work is required to prove a true causal relationship. Overall, the pharmacogenomic approach to behavioral side effects may provide a future tool to predict adverse psychotropic effects related to antiepileptic drugs.**

KEY WORDS: **Levetiracetam, Pharmacogenetics, Aggression, Dopamine, Vulnerability.**

Levetiracetam (LEV) is a second-generation antiepileptic drug (AED) proven to be effective in partial and generalized epilepsy syndromes as sole or add-on medication (Ben-Menachem & Falter, 2000; Berkovic et al., 2007; French & Pedley, 2008). LEV has no relevant interactions with other AEDs and is usually well tolerated in most patients (Lo et al., 2011; Lyseng-Williamson, 2011). Surprisingly, LEV was reported to induce psychotropic side effects in up to 30% of patients. These adverse effects include beneficial effects such as enhancement of drive and cognition on the one hand and behavioral disturbances such as irritability, aggression, agitation, anger, anxiety, apathy, and hostility on the other hand. Especially the negative adverse effects may require discontinuation of LEV treatment (Dinkelacker et al., 2003; Abou-Khalil, 2005).

Apart from its antiepileptic efficacy, the adverse profile of an AED is important when counseling people with chronic epilepsy. Clinical and genetic features predicting the risk for such events would be of outstanding value for the selection of an appropriate and individual treatment regimen. Here, we hypothesized that single nucleotide polymorphisms (SNPs) of genes involved in biologic pathways of aggressive behavior predispose to psychiatric complications due to LEV intake. The idea for this assumption was raised on the basis of behavioral data, which indicated that patients with a personality trait of impulsivity and aggression may be predisposed to show negative behavioral side effects under LEV (Helmstaedter et al., 2008).

To assess the genetic contribution to such a vulnerability, we combined the candidate gene approach, focusing on potential polymorphisms according to their relevance for

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impulsivity and reactive-impulsive aggression (Cordell & Clayton, 2005), with a two-stage association study design to minimize false-positive findings. Finally, to increase statistical power, genetic association was tested through a joint meta-analysis (Skol et al., 2006; Terracciano et al., 2010).

The most severe psychiatric complications of LEV encompass aggressive, hostile, and violent behavior. Aggression is a complex behavioral phenotype often classified as either instrumental-proactive or reactive-impulsive, with the former being goal-directed and characterized by a lack of empathy and remorse and the latter resulting from a loss of inhibitory self-control following anger outbursts (Craig & Halton, 2009). The behavioral phenotype typically observed in patients treated with LEV resembles reactive-impulsive aggression (Helmstaedter et al., 2008). Three degrading enzymes regulating the synaptic activity of the neurotransmitter dopamine have been implicated in this type of aggression: dopamine-β-hydroxylase (DBH) (Rogeness et al., 1982), catechol-*O*-methyltransferase (COMT) (Gogos et al., 1998), and monoamine oxidase (MAO) (Brunner et al., 1993; Cases et al., 1995; Craig & Halton, 2009). Furthermore, the presynaptically and postsynaptically located dopamine receptor D2 (DRD2) has been implicated in disorders characterized by impulsivity and reactive-impulsive aggression, such as addiction, compulsive gambling, or attention-deficit/hyperactivity disorder (ADHD) (Comings et al., 1991; Kirley et al., 2002; Stelzel et al., 2010).

Consequently, stage I had an a priori focus on SNPs located in genes encoding DBH (rs1611115), COMT (rs4680), MAOA (rs6323), and DRD2/ANKK1 (rs1800497, known as TAQ-1A polymorphism). MAOA and COMT also contribute to the degradation of the neurotransmitters norepinephrine (NE) and serotonin (5-HT), both of which have been linked to aggressive behavior (Craig & Halton, 2009). Therefore, to control for the specificity of the neurochemical pathway mediating increased susceptibility to the adverse psychotropic side effects of LEV, further analyses included aggression-related SNPs located in genes regulating noradrenergic and serotonergic signaling: NE transporter 1 (NET1, rs3785143), 5-HT1A receptor (HTR1A, rs6295), 5-HT2A receptor (HTR2A, rs6311), and tryptophan hydroxylase 1 (TPH1, rs1800532 and rs1799913).

Given the hypothesis that psychiatric complications of LEV result from an exacerbation of preexisting (sub)clinical behavioral traits, we predicted that vulnerability to these adverse side effects would be reflected in a specific constellation of risk genes related to impulsivity and reactive-impulsive aggression. We therefore restricted our analysis to candidate SNPs specifically involved in impulsivity and reactive-impulsive aggression. For an overview, Table 1 provides a summary of the functional correlates of the polymorphisms that a priori qualified for this study.

Materials and Methods

Subjects

The stage I study included 298 consecutive patients with chronic epilepsy (including 34 patients with idiopathic generalized epilepsy, 185 patients with symptomatic focal

Table 1. Functional correlates of the polymorphisms examined in stage I

Gene	SNP	Functional correlate(s)	Study
COMT	rs4680	The G allele is associated with increased risk for ADHD (Eisenberg et al., 1999). In ADHD G allele carriers have more symptoms of conduct disorder, are more aggressive, and more likely to be convicted of criminal offenses, compared with A allele carriers (Caspi et al., 2008; Nobile et al., 2010)	Eisenberg et al. (1999) Caspi et al. (2008) Nobile et al. (2010)
MAOA	rs6323	G allelic status is associated with increased risk for ADHD (Domschke et al., 2005; Xu et al., 2007)	Domschke et al. (2005) Xu et al. (2007)
DBH	rs1611115	The TT genotype is associated with increased levels of impulsivity and aggression (Zhang et al., 2005; Hess et al., 2009)	Zhang et al. (2005) Hess et al. (2009)
DRD2/ANKK1	rs1800497	The T allele has been associated with dysregulated impulsivity such as alcohol and tobacco addiction (Munafo et al., 2009) as well as aggressive behavior and conduct disorder (Zai et al., 2011)	Munafo et al. (2007) Zai et al. (2011)
HTR1A	rs6295	The C allele is associated with significantly lower risk for antisocial behavior and conduct disorder in alcohol-dependent subjects (Soyka et al., 2004)	Soyka et al. (2004)
HTR2A	rs6311	CC homozygotes report increased levels of anger- and aggression-related behavior (Giegling et al., 2006)	Giegling et al. (2006)
TPH1	rs1799913	Homozygous A allele carriers showed the highest proactive aggression and homozygous C allele carriers the lowest, whereas measures of reactive aggression did not differ significantly between genotypes (Hennig et al., 2005)	Hennig et al. (2005)
TPH1	rs1800532	Carriers of the A allele reported experiencing unprovoked anger and expressing their anger outwardly more frequently than C allele carriers (Manuck et al., 1999)	Manuck et al. (1999)
NET1	rs3785143	T allele carriers display decreased promoter function associated with higher risk for ADHD and aggressive behavior (Kim et al., 2006; Craig & Halton, 2009)	Craig and Halton (2009) Kim et al. (2006)

epilepsy, 70 patients with cryptogenic focal epilepsy, and 9 patients with undefined epilepsy syndrome) who were under chronic treatment with LEV. The patients' mean age was 38.4 ± (standard deviation) 13.2 years; the male-to-female ratio was 151:147. All patients were receiving long-term treatment with LEV. Nine percent of the patients were taking LEV monotherapy (mean number of antiepileptic drugs, 2.4 ± 0.8). Co-medication was, in descending order, lamotrigine (N = 116), carbamazepine (N = 68), oxcarbazepine (N = 41), valproic acid (N = 36), topiramate (N = 33), phenobarbital (N = 28), pregabalin (N = 24), or phenytoin (N = 10).

The stage II study included an independent sample of 100 patients with chronic epilepsy (three idiopathic generalized epilepsy, 62 symptomatic focal epilepsy, 23 cryptogenic focal epilepsy, 12 not defined epilepsy syndrome) who were under permanent treatment with LEV. Patients' mean age was 38.8 ± 12.4 years; the male-to-female ratio was 58:42. Twenty percent of the patients were receiving LEV monotherapy. Comedication was, in descending order, lamotrigine (N = 26), carbamazepine (N = 21), valproic acid (N = 20), oxcarbazepine (N = 14), topiramate (N = 8), clobazam (N = 7), phenobarbital (N = 4), zonisamide (N = 4), pregabalin (N = 3), phenytoin (N = 3), lacosamide (N = 3), primidone (N = 2), gabapentin (N = 2), clonazepam (N = 2), or eslicarbazepine acetate (N = 1).

The study was approved by the institutional review board of the Medical Faculty of the University of Bonn [NR. 236/04], and all patients provided written informed consent prior to study inclusion.

Behavioral assessment

In the stage I study, data were acquired using structured clinical interviews. Initially, patients were asked to rate psychotropic side effects that they specifically attributed to the intake of LEV on a discrete 5-stepped scale: very negative (−2), negative (−1), no side effects (0), positive (1), or very positive side effects (2). These initial ratings were subsequently specified and validated with use of analog scales on physical well-being, mood, and cognition; the Barratt Impulsiveness Scale; and a German personality questionnaire for patients with central nervous system diseases. Previous analyses showed that ratings of negative behavioral changes expressed loss of self-control, restlessness, sleep problems, and most importantly, reactive-impulsive aggression, whereas positive ratings expressed increased energy and concentration (Helmstaedter et al., 2008). Notably, ratings of increased aggression under LEV were found to be highly correlated with 149 proxy reports from significant others who underwent the same interview and got the same questionnaires and scales (r = 0.65). Given its reliability and validity, the five-stepped rating of behavioral changes was employed as the outcome measure in the subsequent statistical analysis.

Data of the stage II study were acquired from clinical records, according to a predefined protocol: for each patient clinical records were screened for the presence versus absence of negative psychotropic side effects of LEV treatment. Data acquisition thus resulted in a dichotomous outcome score: LEV treatment caused negative psychotropic side effects (1) versus LEV did not cause such effects (0). This score was used as the outcome measure in subsequent statistical analyses.

Genetic analysis

Whole-genomic DNA was extracted from 10-ml aliquots of ethylenediaminetetraacetic acid (EDTA)-anticoagulated blood using a salting-out method (Miller et al., 1988). Genotyping for the single nucleotide variants rs12364283, rs1611115, rs1799913, rs1800532, rs3785143, rs6295, rs6311, and rs6323 was performed by pyrosequencing on a PSQ HS96A instrument (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Genotyping of rs1800497 and rs4680 was performed using the commercially available TaqMan assays C_7486676_10 and C_25746809_50 on a 7900HT fast realtime PCR system (Applied Biosystems, Foster City, CA, U.S.A.) according to the manufacturer's instructions. Several positive and negative controls were analyzed to exclude mix-ups, contaminations, and other errors during genotyping. Primer sequences and assay conditions for the noncommercial pyrosequencing assays are available from the authors upon request.

Data analysis

For the stage I study, single and multiple SNP analyses were carried out using R 2.9.2 (R Development Core Team, Vienna, Austria) and SNPStats (Sole et al., 2006). For all SNPs, genotype frequencies, Hardy-Weinberg equilibrium (HWE), and linkage disequilibrium (LD) were determined. Associations between genotypes and psychotropic side effects, as well as interactions with sex, as a covariate, were tested by linear regression models. Multiple inheritance models (codominant, dominant, and recessive) were employed, with Akaike Information Criterion (AIC) as a measure of model fit. Estimated p-values were used to rank SNPs according to their statistical relevance, and the top-ranked ones ($p < 0.05$) were taken forward to stage II. The extent to which these SNPs discriminated between high and less susceptible patient groups in stage I was quantified through Hedges' g, an unbiased estimator of effect size. Hedges' g-values were also used to assess the increase in the load of negative psychotropic side effects for carriers of multiple susceptibility–associated alleles.

Top-ranked effects obtained from stage I ($p < 0.05$) were tested with directed hypotheses in stage II by means of chi-square tests for 2×2 contingency tables. For each SNP only the inheritance model with the highest statistical significance as determined in stage I was tested. This procedure

increased statistical power while avoiding the critical issue of multiple testing in stage II.

Finally, a sample-weighted r-based random effects meta-analysis method was used to combine results from both stages (using the packages ''compute.es'' and ''MAc'' for R 2.9.2). Therefore, for each stage, a separate r-statistic summarizing the magnitude of its association with negative psychotropic side effects and the direction of this association was generated for each SNP entering stage II (Skol et al., 2006; Terracciano et al., 2010). An overall r-statistic was then computed as a weighted average of the associations observed in both stages (Terracciano et al., 2010). For each average r-statistic, a corresponding p-value reflecting statistical significance and a Q-value proportional to the heterogeneity of effects between both stages was computed. Because three SNPs entered stage II, a Bonferroni corrected p-value < 0.0167 was considered significant.

Results

Stage I study

The call rates for the genotyped SNPs were in the range of 98.6–100% (Table S1). The genotype distributions of three SNPs deviated from HWE (Tables S2 and S3): COMT (rs4680, p = 0.048) and DBH (rs1611115, p = 0.017) (rs6323 was omitted from HWE analysis in the mixed male/female patient sample since MAOA maps on the X chromosome). Similar distributions for these SNPs were reported for the Central European collection (CEU) of the International HapMap Project (http://hapmap.ncbi.nlm.nih.gov/). A chi-square test yielded no differences (p > 0.05), except for the distribution of rs1611115(T;T) genotypes, which were more frequent in the CEU, as compared to our patient sample (5% and 1%, respectively, p = 0.0027). However, this result might have been caused by the low frequency of this genotype (five patients in the CEU sample and four patients in our sample). Statistical analysis yielded no significant LD (all p-values > 0.05).

Psychotropic side effects in either direction (positive/negative) were reported by 60% of patients (rated as very negative by 12%, negative by 26%, positive by 15%, and very positive by 7% of the patients), and 19% of patients were classified as nonresponders. Three SNPs were associated with adverse psychotropic side effects (Table 2). Patients with elevated degrading DBH activity (rs1611115(C;C) genotype) reported higher levels of adverse side effects (Table 2) in the codominant (p = 0.018, difference = 0.36, CI = 0.11–0.61) and dominant (p = 0.007, difference = 0.34, CI = 0.09–0.59) models (Fig. 1A). Furthermore, an interaction was found between COMT (rs4680) and sex: male, but not female, patients with increased degrading COMT activity (rs4680(G;G) genotype) reported a higher load of negative side effects (p = 0.0045; Fig. 1B,C). In addition, DRD2/ANKK1 TAQ-1A A+ genotype (the T allele of rs1800497) was associated with increased susceptibility to negative side effects (Table 2) when a dominant model was applied (p = 0.041, difference = −0.28, CI = −0.54 to −0.1; Fig. 1D). The abovementioned SNPs were associated with an 8.2–19.3% increase in the rate of LEV treatment-induced psychotropic

Table 2. Stage I study: Associations between SNPs and side effects of LEV treatment

SNP (Gene)	Model	Genotype	N	Response mean (SEM)	Difference (95% CI)	p-Value (two-tailed)	AIC
rs4680; men (*COMT*)	Codominant	A/A	46	−0.17 (0.14)	0	**0.016**	430.5
		A/G	60	−0.08 (0.13)	−0.09 (−0.31 to 0.49)		
		G/G	41	−0.66 (0.18)	−0.48 (−0.92 to −0.05)		
	Dominant	A/A	46	−0.17 (0.14)	0	0.440	436.1
		A/G-G/G	101	−0.32 (0.11)	−0.14 (−0.51 to 0.22)		
	Recessive	A/A-A/G	106	−0.12 (0.1)	0	**0.0045**	428.7
		G/G	41	−0.66 (0.18)	−0.54 (−0.91 to −0.17)		
rs1611115 (*DBH*)	Codominant	C/C	176	−0.36 (0.08)	0	**0.018**	840.3
		C/T	106	0 (0.1)	0.36 (0.11 to 0.61)		
		T/T	4	−0.5 (0.87)	−0.14 (−1.18 to 0.89)		
	Dominant	C/C	176	−0.36 (0.08)	0	**0.007**	839.2
		C/T-T/T	110	−0.02 (0.1)	0.34 (0.09 to 0.59)		
	Recessive	C/C-C/T	282	−0.22 (0.06)	0	0.600	846.1
		T/T	4	−0.5 (0.87)	−0.28 (−1.32 to 0.76)		
rs1800497 (*DRD2/ANKK1*)	Codominant	C/C	203	−0.14 (0.08)	0	0.12	858.8
		T/C	78	−0.42 (0.1)	−0.29 (−0.56 to −0.01)		
		T/T	9	−0.33 (0.24)	−0.20 (−0.90 to 0.51)		
	Dominant	C/C	203	−0.14 (0.08)	0	**0.041**	856.8
		T/C-T/T	87	−0.41 (0.09)	−0.28 (−0.54 to −0.01)		
	Recessive	C/C-T/C	281	−0.22 (0.06)	0	0.750	860.9
		T/T	9	−0.33 (0.24)	−0.12 (−0.82 to 0.59)		

SNP, single-nucleotide polymorphism; since the effect of rs4680 interacted with sex, analysis results are presented for men only (in women all p-values > 0.05); for all other SNPs values indicate the effect in both sexes; N, number of subjects; HWE, Hardy-Weinberg equilibrium; bold indicate p-values < 0.05.

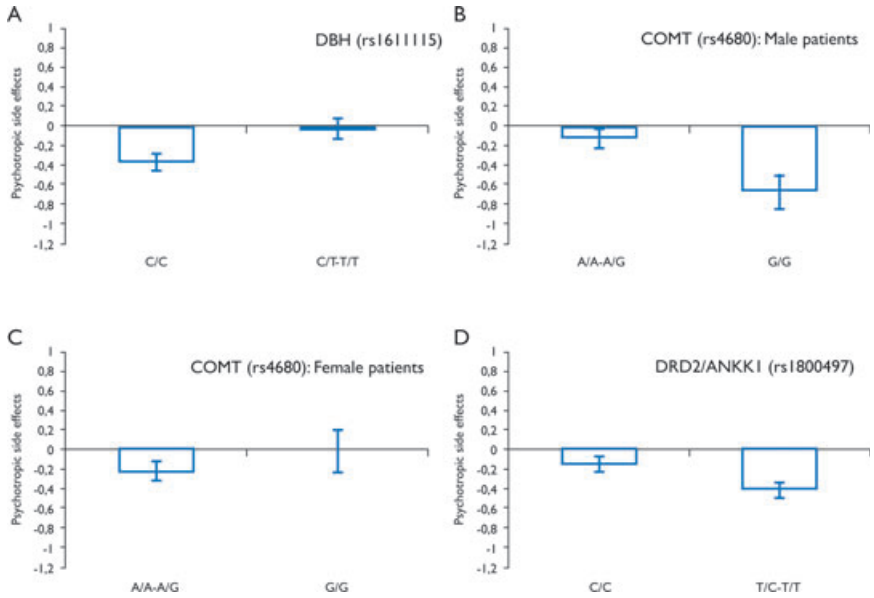

Figure 1.
SNPs associated with negative psychotropic side effects of LEV treatment in stage I. (**A**) Increased DBH activity (homozygous carriers of the C allele of rs1611115) was associated with a higher load of negative side effects (p = 0.007); In male (**B**) but not female (**C**) patients, increased activity of the dopamine degrading enzyme COMT (homozygous carriers of the G allele of rs4680) was associated with a higher load of negative psychotropic side effects (p = 0.0045); (**D**) low synaptic availability of the dopamine D2 receptor (the T allele of the DRD2/ANKK1 TAQ-1A polymorphism rs1800497) was associated with a higher load of negative side effects (p = 0.041).
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side effects (Table S5). Apart from this, no further associations were observed (all p-values > 0.05, Table S4).

Genetic modulators of NE and 5-HT signaling were not found to be associated with the side effects of LEV treatment (p-values > 0.05 for all effects and all interactions; Table S6). Therefore, the recessive inheritance model for rs4680 (COMT, in men) and the dominant inheritance models for rs1611115 (DBH) and rs1800497 (DRD2/ANKK1) were nominated for stage II.

It is intriguing that the load of adverse psychotropic side effects increased for carriers of multiple susceptibility–associated alleles. The effects of DBH and DRD2/ANKK1 cumulated (Table 3) such that patients with elevated degrading DBH activity and lower DRD2 expression (rs1611115(C;T) & rs1800497(C;C)-genotype) reported a higher load of adverse psychotropic side effects than patients with lower degrading DBH activity and higher DRD2 expression (rs1611115(C;C) & rs1800497(T;C)-genotype): Hedges' g = 0.587. Furthermore, the effects of DBH, COMT, and sex cumulated: Hedges' g = 1.46 (Table 3). Therefore, male patients with increased activity of both DBH and COMT (rs1611115(C;C) and rs4680 (G;G)-genotype) reported the highest load of negative side effects in stage I.

Stage II study

Adverse psychotropic side effects were reported for 23% of patients. Among the SNPs nominated for stage II, no

Table 3. Stage I study: Interactions among SNPs and between SNPs and sex

SNP	Contrast	Hedges' g	N (n 1; n 2)	% total (%n1; %n2)
DRD2	C/C – T/C	0.255	281 (203; 78)	94 (68; 26)
DBH	C/C – C/T	0.322	282 (176; 106)	95 (59; 36)
COMT*Sex	Men only: (A/A-A/G) – G/G	0.507	147 (106; 41)	49 (36; 14)
DBH*DRD2	(C/T-C/C) – (C/C-T/C)	0.587	138 (85; 53)	46 (28; 18)
DBH*COMT	(C/T-G/G) – (C/C-G/G)	0.876	67 (21; 46)	22 (7; 15)
DBH*COMT*Sex	Women(C/T-G/G) – Men(C/C-G/G)	1.462	36 (10; 26)	12 (3; 9)

SNP, single-nucleotide polymorphisms; N, total number of subjects in both groups; n1, number of subjects in the first group; n2, number of subjects in the second group; %total, percentage of the total sample size (298 patients), represented by both groups; %n1, percentage of the total sample size (298 patients), represented by the first group; %n2, percentage of the total sample size (298 patients), represented by the second group; DRD2, rs1800497; DBH, rs1611115; COMT, rs4680.

deviations from HWE were found (all p-values > 0.05, Table S7). Neither the effect of DBH (χ^2 = 0.043; p = 0.418), nor the effect of COMT in males (χ^2 = 0.017; p = 0.448) observed in stage I could be replicated in stage II. However, the trend for increased risk of negative psychotropic side effects for the DRD2/ANKK1 TAQ-1A A+ genotype, as observed in stage I, was confirmed (χ^2 = 1.845; p = 0.0872) (Table 4). Specifically, the rate of adverse side effects was 30% for T allele carriers (A+ genotype) and 18.3% for homozygous C allele carriers (Table S8).

Joint meta-analysis

The strongest and most homogeneous meta-analytic signal was found for DRD2/ANKK1 (r = 0.131; Q = 0.002; p = 0.0096), which survived correction for multiple comparisons (Table 5). Although the mean effect size for DBH and COMT was comparable, Q-values indicated that their effects were highly heterogeneous across both stages, such that mean effect size scores were biased by the observations in stage I and not statistically significant (r = 0.116, Q = 1.531, p = 0.0892 for DBH; r = 0.160, Q = 2.324, p = 0.1740, for COMT in males; Table 5). If r2 is considered as an estimator of explained variance, then the DRD2/ANKK1 TAQ-1A genotype can explain between 1.7% (95% confidence interval: 0.1–5.2%) of the total LEV adverse side effect variance. In view of the binomial effect size display (Rosenthal & Rubin, 1982), this effect size corresponds to 13.2% change (95% confidence interval: 3.2–23%) in adverse side effect rate attributable to LEV.

Discussion

Following the hypothesis that patients may be predisposed to become irritated or aggressive under the treatment with LEV, we have investigated the association between negative psychotropic side effects with LEV treatment and nine different SNPs related to aggressive behavior. Three of the investigated SNPs (rs1611115, rs4680, and rs1800497) were significantly associated with negative psychotropic effects in the stage I population. However, only the rs1800497 SNP was, though only weakly, confirmed in the independent stage II population and the subsequent joint meta-analysis. This novel finding suggests a link between dopaminergic activity and negative psychotropic effects with the use of LEV.

Mechanisms involved in aggressive behavior in the context of LEV intake

At present, we do not fully understand the precise cellular mechanisms mediating the central nervous system effects of LEV, including its antiepileptic action and negative psychotropic side effects. Unlike other antiepileptic drugs, LEV has a high binding affinity to SV2A (synaptic vesicle glycoprotein 2A), a presynaptic protein involved in synaptic exocytosis, and has been shown to block presynaptic calcium channels as well as postsynaptic AMPA-type glutamate

Table 4. Stage II study: Associations between genetic modulators of DA signaling and the side effects of LEV treatment

SNP (Gene)	Genotype	N (side effects)	Side effects (%)	χ^2 (chi-square)	p-Value (one-tailed)
rs4680; men (COMT)	A/A-A/G	41 (9)	22	0.017	0.4478
	G/G	17 (4)	23.5 (1.5% increase)		
rs1611115 (DBH)	C/T-T/T	41 (9)	22	0.043	0.4177
	C/C	59 (14)	23.7 (1.7% increase)		
rs1800497 (DRD2/ANKK1)	C/C	60 (11)	18.3	1.845	0.0872
	C/T-T/T	40 (12)	30.0 (11.8% increase)		

SNP, single-nucleotide polymorphisms; N, number of subjects; χ^2, Pearson's chi-square.

Table 5. Meta-analytic analysis of association across stage I and stage II

SNP (Gene)	Model	Genotype	r	r lower	r upper	Q	p-Value (two-tailed)
rs4680; men (*COMT*)	Recessive	A/A-A/G G/G	0.1600	−0.0711	0.3744	2.3240	0.1740
rs1611115 (*DBH*)	Dominant	C/C C/T-T/T	0.1164	−0.0179	0.2465	1.5310	0.0892
rs1800497 (*DRD2/ANKK1*)	Dominant	C/C T/C-T/T	0.1314	0.0321	0.2281	0.0026	0.0096

SNP, single-nucleotide polymorphism; r, overall effect size for the meta-analysis; r lower, lower 95% confidence interval; r upper, upper 95% confidence interval; Q-statistic, measure of heterogeneity; the bold indicates the significant result after Bonferroni correction for multiple comparisons.

receptors (Surges et al., 2008). Therefore, it is possible that modulation of these targets by LEV interacts with dopamine neurotransmission and neural circuits initiating and/or disinhibiting aggressive behavior. Our data suggest that LEV specifically interferes with dopaminergic neurotransmission. The DRD2/ANKK1 TAQ-1A polymorphism rs1800497 is a missense mutation located 9,489 base pairs downstream from the 3′ end of DRD2, within a gene coding for ankyrin repeat and protein kinase domain-containing protein 1 (ANKK1). Due to a strong linkage disequilibrium with DRD2, it can be considered a reliable marker for variation in D2 receptor synaptic availability: carriers of the T allele show a 30% reduction of D2 binding sites, compared to homozygous (C;C) carriers (Ritchie & Noble, 2003). This effect is particularly prominent in the striatum, but also extends to the prefrontal cortex, which is involved in the top-down regulation of anger and impulsivity. Our analyses revealed a highly homogeneous effect of TAQ-1A across both study stages. Specifically, we show that the T allele defines a risk allele for the negative psychotropic side effects of LEV. In addition to its contribution to the adverse psychiatric profile of LEV shown here, the TAQ-1A T+ genotype has been implicated in disorders characterized by dysregulated impulsivity, such as alcohol and tobacco addiction (Munafo et al., 2009), compulsive gambling, and attention-deficit/hyperactivity disorder (Stelzel et al., 2010). Furthermore, T allelic status has been associated with the personality traits neuroticism and depression (Kestler et al., 2000; Kazantseva et al., 2011), as well as with aggressive behavior and conduct disorder in children (Zai et al., 2011). Because all top-ranked genes from stage I were associated with reduced synaptic dopamine activity (Fig. 2), and in view of the major role of dopamine in impulsivity, the effect of DRD2/ANKK1 TAQ-1A we suggest is specifically mediated through alterations in dopamine signaling.

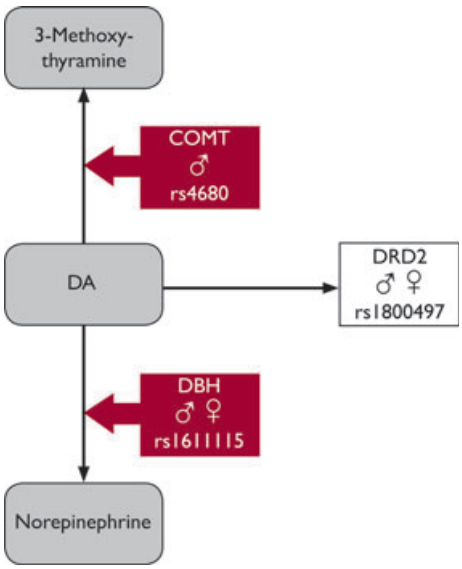

Figure 2.
Reduced dopaminergic activity is associated with increased susceptibility to the negative psychotropic side effects of LEV (stage I). Lower dopamine activity, as determined by lower expression of dopamine D2 receptors, increased activity of the dopamine degrading enzyme COMT and increased DBH activity, was associated with higher loads of negative side effects in stage I of our study. The effect of COMT was present in male, but not female patients (♂ sign), whereas DBH and DRD2 modulated side effects for both sexes (♂♀ sign).
Epilepsia © ILAE

Study strengths and limitations

A limitation of the present study may be seen in that fact that this study followed a candidate gene approach focusing on SNPs specifically involved in impulsivity and reactive-impulsive aggression. However, the selection of the candidate genes was not arbitrary but hypothesis-driven. It cannot be excluded that we have missed other SNPs and pathways that are important for the negative psychotropic effects associated with LEV intake. In addition, it would be of clinical interest to investigate the genetic basis of pharmacoresistance to LEV or the relationship between seizure-control and occurrence of adverse effects. For instance, a recent study has found no association between pharmacoresistance to LEV and common variations of its putative major target SV2A (Lynch et al., 2009). These questions were beyond the scope of this evaluation and have not been addressed in the present manuscript.

The most important limitations of our study are the relatively weak p-values in the confirmatory stage II population and the joint meta-analysis. However, although the effect of DRD2/ANKK1 TAQ-1A had a p-value of 9.6×10^{-3} only and thus ranged below the threshold employed in genome-wide association studies (Terracciano et al., 2010), it still remained significant after Bonferroni-correction. In our view this reflects the efficiency of the evidence-based and hypothesis-driven candidate gene-approach adopted in the present study: previous findings (Helmstaedter et al., 2008) of increased impulsivity in patients at higher risk led us to select nine impulsivity-related polymorphisms, one third of which were associated with psychiatric complications in stage I. Of these three, TAQ-1A was the only polymorphism yielding significance in the final joint meta-analysis. The effects of DBH and COMT might be more variable and heterogeneous per se, perhaps requiring much larger samples to be replicated with sufficient power (Skol et al., 2006). Furthermore, the type of data acquisition differed in the initial and the replication study. Prospective structured clinical interviews of patients and relatives in stage I can be suggested to result in more precise information than and post hoc examination of clinical reports in stage II. Connected to this in addition the scaling in stage I (five categories ranging from positive to negative) and stage II (binary negative vs. not negative) was different. Hence different data quality

might have interacted with effect size distribution, further increasing the effect size variability. Certainly, use of the same dependent measures in the initial and the replication study could have provided more statistical power. However, the highly homogeneous effects of DRD2/ANKK1 TAQ-1A despite different assessments of the behavioral outcome corroborate the validity of this finding.

Finally, it should be taken into account that the samples examined in this study were relatively small and that the present data provide first evidence of genetic risk factors for psychotropic side effects of LEV, which needs to be further substantiated by subsequent studies.

Conclusions

In summary, we present first evidence that risk variants in aggression-related genes regulating dopaminergic but not serotonergic or noradrenergic signaling are associated with the adverse psychiatric profile of LEV. Decreased synaptic availability of D2 receptors, associated with the DRD2/ANKK1 TAQ-1A T+ genotype, defines a subgroup of patients with a higher load of negative psychotropic side effects. Replication and further studies are required to demonstrate a true causal relationship. Overall, our study is in line with a promising, yet nascent, approach in pharmacogenetic research (McCormack et al., 2011): characterizing the genetic underpinnings of behavioral profiles for antiepileptic drugs may help to optimize treatment choices by minimizing severe psychiatric complications and thus significantly improve patients' quality of life.

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Disclosure

C.H. served on scientific advisory board of UCB and Desitin, received honoraria for biostatistical data analyses and publications from Eisai, UCB, and Desitin, and received license fees from UCB. R.S. serves on the scientific advisory board of UCB, served on the speaker's Bureau for Eisai, and has received funding for travel from Eisai. C.E.E. is consultant for Desitin und Novartis and received honoraria for talks from Pfizer and Esai. H.T., P.N., M.R.T., Y.M., S.S., W.S.K., and R.H. have no conflicts of interest. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Call rates for the SNPs in stages I and II.

Table S2. Stage I: Genotype distributions of aggression-related SNPs, modulating dopamine signaling.

Table S3. Stage I: Genotype distributions of aggression-related SNPs, modulating norepinephrine and 5-HT signaling.

Table S4. Stage I: Associations between genetic modulators of DA signaling, and the side effects of LEV treatment.

Table S5. Stage I: Rates of LEV side effects for the top-ranked polymorphisms.

Table S6. Stage I: Associations between genetic modulators of norepinephrine and 5-HT signaling, and the side effects of LEV treatment.

Table S7. Stage II: Genotype distributions of SNPs.

Table S8. Stage II: Rates of LEV side effects for the tested polymorphisms.

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