to filter for potential phenotype-relevant target genes. After cloning 3'-UTR sequences containing predicted miRNA binding sites into psiCHECK2 or pmiRGLO vectors reporter gene assays were performed to confirm RNA interference of selected candidate miRNAs with their respective target genes.
Results: Of 754 miRNAs, 201 were detected in both tissue types. Two miRNAs were differentially expressed in the hippocampus relative to the cortex (miR-34c-5p: 7.2-fold higher [q = 0.01], miR-212-3p: 3.8-fold lower [q = 0.01]). Bioinformatic analysis and filtering for target genes identified 9 genes important for drug efflux, neuronal regulation, and signal transmission. Reporter gene experiments confirmed 3 target genes posttranscriptionally regulated by miR-34c-5p (GABBR2, GABRA3, GRM7) and 3 target genes regulated by miR-212 (ABCG2, SOX11, ADCY1).
Conclusion: Differential regulation of 2 miRNAs could contribute to an altered function of several genes resulting not only in an imbalanced neuronal excitability but also in impaired neural differentiation and accelerated drug export. These data suggest multifactorial alterations involving miRNA-mediated regulation leading to pharmacoresistance in epilepsy.
Financial Sources: This work was supported by a fellowship from DFG (Ha 6112/1-1) and NIH grant GM61390.
Disclosure of Interest: None declared.

PP124—PHARMACOKINETICS OF TOLPERISONE IN RELATION TO CYP2C19 GENOTYPES

J.-Y. Byeon*; J.-Y. Lee; J.-S. Jeon; J.-E. Lee; S.-H. Kim; C.-G. Jang; and S.-Y. Lee
Laboratory of Pharmacology, School of Pharmacy, Sungkyunkwan University, Suwon, Korea, Republic Of
Introduction: Tolperisone, which is indicated in the treatment of acute muscle spasms in back pain and spasticity in neurologic diseases, is a centrally acting muscle relaxant. Although the metabolism of tolperisone is primarily mediated by CYP2D6, CYP2C19, CYP1A2, and CYP2B6 are also involved in the biotransformation of tolperisone. Among 3 drug-metabolizing enzymes, CYP2C19 is a highly polymorphic enzyme. The aim of this study was to investigate the effects of CYP2C19 genetic polymorphism on the pharmacokinetics of tolperisone.
Patients (or Materials) and Methods: Twenty-six healthy Korean subjects were selected and divided into 3 different groups according to CYP2C19 genotype, CYP2C19EM (CYP2C19*1/*1, n = 12), CYP2C19IM (CYP2C19*1/*2 or *1/*3, n = 7), and CYP2C19PM (CYP2C19*2/*2, *2/*3 or *3/*3, n = 7). After overnight fasting, each subject received a single 150-mg oral dose of tolperisone. Blood samples were collected up to 12 hours after drug intake, and plasma concentrations of tolperisone were measured by using LC-MS/MS analytical system.
Results: Cmax in CYP2C19PM group was significantly higher than that in CYP2C19IM and CYP2C19EM ($P = 0.0017$ for all). AUCinf in CYP2C19PM was also significantly higher than that in CYP2C19IM and CYP2C19PM group ($P < 0.001$ for all). Corresponding values for tolperisone in CYP2C19EM and IM groups were almost similar ($P > 0.05$). Apparent oral clearance (CL/F) of tolperisone in CYP2C19PM group was 84% lower than that in CYP2C19IM group (618 [379] vs 2900 [1343] L/h; $P = 0.0010$). Differences in other parameters of tolperisone between 3 genotype groups were not statistically significant.
Conclusion: In Korean healthy subjects, pharmacokinetics of tolperisone are not only influenced by CYP2D6 genotypes but also influenced by CYP2C19 genotypes. Particularly, CYP2C19PM subjects had markedly increased plasma concentration of tolperisone compared with CYP2C19EM or CYP2C19IM subjects.
Disclosure of Interest: None declared.

PP126—IN-VITRO REACTIVITY OF DRUG-SPECIFIC T-CELLS FROM A HLA-A*31:01 POSITIVE CARBAMAZEPINE HYPERSENSITIVE PATIENT

M. Lichtenfels*; D.J. Naisbitt; A. Alfirevic; K.B. Park; and M. Pirmohamed
Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, United Kingdom
Introduction: Carbamazepine (CBZ) causes hypersensitivity reactions in a small proportion of patients. There is strong evidence that specific human leukocyte antigen (HLA) alleles are associated with a higher risk of developing CBZ-induced hypersensitivity. HLA-A*3101 represents the latest example and is associated with several clinical phenotypes of CBZ-induced hypersensitivity in Caucasian and Japanese patients. In this study, we aimed to determine whether HLA-A*31:01 is functionally implicated in the development of a drug-specific immune response in our patient.
Patients (or Materials) and Methods: Peripheral blood mononuclear cells (PBMCs) were isolated from a patient with CBZ hypersensitivity and the presence of drug-responsive T cells confirmed in vitro using the lymphocyte transformation test (LTT). The study was approved by the local ethics committee and informed consent was obtained from the patient. Drug-specific T cells were enriched in a 4-week induction culture and their reactivity tested with enzyme-linked immunospot (ELISpot) technique and 51Cr-release assay. T-cell clones (TCCs) were generated by serial dilution; characterization included CD phenotype, HLA restriction and cytokine profile.
Results: PBMCs responded to CBZ in the LTT with a stimulation index (SI) of 15.9. After the 4-week enrichment culture, T-cells were shown to secret Interferon-γ (IFN-γ) and kill 51Cr-loaded target cells when exposed to CBZ. Thirty-two CBZ-specific TCCs were generated; they secreted IFN-γ, interleukin-13 and cytolytic molecules such as granzyme B, perforin, and FasLigand. The majority of TCCs were CD4+ and T-cell activation was restricted by HLA class II alleles, i.e. HLA-DR and -DP. These TCCs proliferated in the presence of both CBZ and antigen presenting cells (APCs) expressing HLA-A*31:01 and HLA-DRB1*04:04, but also in the presence of HLA-DRB1*04:04+ APCs lacking HLA-A*31:01. HLA-DRB1*04:04 is known to be part of a common haplotype with HLA-A*31:01 in Caucasians.
Conclusion: We were able to stimulate a secondary immune response to CBZ in vitro using lymphocytes from a HLA-A*31:01+ hypersensitive patient. CBZ-specific T cells of CD4+ phenotype were restricted by HLA class II alleles, and proliferated in the presence of CBZ and HLA-DRB1*04:04+ A*31:01- APCs revealing that a common haplotype may contribute to the multi-clonal response seen in patients with CBZ hypersensitivity. Further studies are needed to confirm the association.
Disclosure of Interest: None declared.

PP127—CYP4F2 AND APOE CONTRIBUTION IN ACENOCOUMAROL DOSING BASED ON GENOTYPE: A COMPARISON OF TWO ALGORITHMS

H.Y. Tong*; A.M. Borobia; R. Lubomirov; C. Zegarra; S.H. Lei; J. Frías; and A.J. Carcas
Clinical Pharmacology, La Paz University Hospital, Madrid, Spain
Introduction: Two algorithms for acenocoumarol stable dose prediction have been recently published. The first, developed by the EU-PACT group includes demographic (age, sex, weight, height, amiodarone cotreatment) and genetic variables (genetic variants in *CYP2C9* and *VKORC1* genes). The second one has been developed by our group (HULP algorithm) in a cohort of 147 patients with thromboembolic disease (VTD), including clinic-demographic

(age, BMI and presence of amiodarone or metabolic inducers) and genetic variables (genetic variants in *CYP2C9, VKORC1, CYP4F2* and *APOE* genes). Our objective was to test the performance of the EU-PACT algorithm in our cohort of patients. Assess the influence of factors included in our algorithm but not considered in the EU-PACT algorithm, specifically *CYP4F2* and *APOE* variants, and concomitant use of metabolic inducers.

Patients (or Materials) and Methods: We evaluated the performance of the EU-PACT algorithm in our cohort (HULP) using coefficient of determination (R_2). To investigate the contribution of vatiants in *CYP4F2* and *APOE*, and concomitant metabolic inducers, we compared the real acenocoumarol doses of patients with these variables and those doses predicted by both models. A third model was built using as independent variables the dose predicted using EUPACT algorithm in our cohort, *APOE* and *CYP4F2* variants, and concomitant metabolic inducers. Paired McNemar's test was used to compare both R_2.

Results: Variability explained by the EU-PACT's algorithm when applied to our (HULP) cohort was 44.4%. The real mean dose in patients with at least 1 of the evaluated variables (*CYP4F2*, *APOE* variants or metabolic inducers) was 19.3 (8.1) mg/week while the dose calculated by the HULP-algorithm was almost the same (19.0 [5.4] mg/week) and the EU-PACT's model underestimates almost 3 mg (16.0 [6.2] mg/week), $P < 0.001$. The third model, evaluating the contribution of *CYP4F2* and *APOE* variants and concomitant metabolic inducers, is able to increase the R_2 from a 44.4% observed with the EU-PACT algorithm to 47.5% ($P < 0.05$).

Conclusion: EU-PACT shows a reasonable performance in an independent cohort with VTD. Inclusion of other known genes involved in high dose requirements as CYP4F2 and ApoE and enzyme inducer drugs, all included in HULP algorithm, seems to improve prediction in acenocoumarol dosing.

Disclosure of Interest: None declared.

PP128—IMPACT OF VARIABILITY IN THE BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) GENE IN EATING DISORDER PATIENTS

C. Gamero[1]; I. Flores[2]; J.A. Carrillo[1]; A. Garcia-Herraiz[2]; I. Gordillo[1]; M. Monge[2]; M. Jimenez[1]; R. Rodriguez-Lopez[3]; and G. Gervasini[1*]

[1]Medical & Surgical Therapeutics, University of Extremadura; [2]Eating Disorders Unit, Health Service of Extremadura; and [3]Genetics Unit, Infanta Cristina University Hospital, Badajoz, Spain

Introduction: Eating behavior has been shown to be affected by the brain-derived neurotrophic factor (BDNF). In the present work, we have aimed to investigate whether *BDNF* genetic variability may influence physiological and psychopathological features in patients with eating disorders (ED) and/or modulate the risk for the disorder.

Patients (or Materials) and Methods: One hundred twenty unrelated female patients with anorexia or bulimia nervosa (AN, BN) and 125 healthy controls were genotyped for *BDNF* single nucleotide polymorphisms (SNPs). Associated psychopathological characteristics were assessed by the EDI-2 and SCL-90R inventories.

Results: With regard to physiological parameters, the rs16917237 TT genotype was associated with increased minimum weight (60.6 [19.3] vs 50.4 [11.9] kg; $P < 0.05$) and BMI (23.3 [7.9] vs 19.3 [4.1]; $P < 0.05$) in the whole population of patients with ED. The risk study showed that only the rs11030119 AA genotype increased the risk for AN (OR = 5.23 [1.32–20.98], p = 0.02), although the association lost significance after Bonferroni correction. AN patients who harbored the -270CC genotype scored higher than CT carriers in the Interpersonal Distrust scale of the EDI-2 questionnaire (6.9 [4.4] vs 2.9 [3.6]; Bonferroni $P < 0.05$). In the same manner, carriers of rs10835210 CC wildtype genotype showed higher scores for the Drive for Thinness scale (13.5 [5.0] vs 9.6 [6.0] for patients with the variant allele; Bonferroni $P < 0.05$). Finally, the haplotype study showed that 2 combinations (haplotypes **4* and **7*) showed significantly higher scores in several scales of the EDI-2 and SCL-90R inventories than those with the most common haplotype **1*.

Table. Descriptive and clinical variables of patients with anorexia nervosa (AN) or bulimia nervosa (BN) and healthy controls. Mean (SD) values are shown.

	AN	BN	Controls	*P*
Age, years	25.4 (7.67)	26.88 (6.88)	22.18 (6.13)	NS
Height, m	1.61 (0.07)	1.61 (0.05)	1.62 (0.05)	NS
Weight, kg	45.56 (6.44)	60.16 (3.84)	58.1 (9.33)	<0.05a
BMI, kg/m^2	17.6 (2.31)	22.88 (5.27)	22.13 (3.45)	<0.05a
Age at onset,	17.16 (5.07)	19.57 (6.7)		
Mean duration of illness, y	19.63 (7.25)	19.62 (5.9)		
Total EDI-2 score	87.46 (42.67)	115.64 (41.88)		
GSI (SCL-90R) score	1.54 (0.81)	1.97 (0.89)		
PST (SCL-90R) score	60.72 (20.7)	71.02 (17.8)		
PSDI (SCL-90R) score	2.17 (0.75)	2.41 (0.57)		

Conclusion: Variability in the *BDNF* gene locus may contribute to psychopathological features that are commonly found in ED patients.

Financial Sources: This work has been done in part with grant GR10022 from Junta de Extremadura (Merida, Spain) and a grant from Fundación Alicia Koplowitz (Madrid, Spain)

Disclosure of Interest: None declared.

PP129—TIME-TO-ACHIEVE STABILITY OF ANTICOAGULATION IS DECREASED IN ABCB1 MUTATED PATIENTS TREATED WITH ACENOCOUMAROL

V. Rollason[1*]; L. Gschwind[1,2]; Y. Daali[1]; F. Boehlen[3]; M. Rebsamen[4]; C. Combescure[5]; A. Matthey[1]; P. Bonnabry[2,6]; P. Dayer[1]; and J.A. Desmeules[1]

[1]Division of Clinical Pharmacology and Toxicology, Geneva University Hospitals, Genève 14; [2]School of pharmaceutical sciences, University of Geneva / University of Lausanne, Geneva/ Lausanne; [3]Division of Angiology and Haemostasis; [4]Division of Laboratory Medicine; [5]Division of Clinical Epidemiology; and [6]Pharmacy, Geneva University Hospitals, Genève 14, Switzerland

Introduction: Acenocoumarol is an oral anticoagulant of the coumarin type. These anticoagulants have a narrow therapeutic index and are characterized by a large interpatient variability that can be explained by numerous factors, including genetic factors. Dose variability due to polymorphisms in the *VKORC1* and the *CYP2C9* genes has been well characterized. The aim of our study was to investigate the potential association between *ABCB1* polymorphisms and the time-to-achieve stability during acenocoumarol treatment.

Patients (or Materials) and Methods: We conducted a prospective observational study on 115 hospitalized patients, aged 18 years and over and starting acenocoumarol. Collected data included sex, age, anticoagulant indication, INR measurements, acenocoumarol doses, comorbidities, comedications, and genotype (*ABCB1* c.3435C>T and c.2677G>T/A). Patients were followed from the date of the first acenocoumarol administration until time-to-achieve stability or the end of the observation period of 35 days. Time-to achieve stability was defined as the first 3 consecutive INR measurements within the