

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.

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### PP124—PHARMACOKINETICS OF TOLPERISONE IN RELATION TO CYP2C19 GENOTYPES

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**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

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**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\*31:01 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 secrete Interferon- $\gamma$  (IFN- $\gamma$ ) and kill 51Cr-loaded target cells when exposed to CBZ. Thirty-two CBZ-specific TCCs were generated; they secreted IFN- $\gamma$ , 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

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**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