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GWAS identifies two susceptibility loci for lamotrigine-induced skin rash in patients with epilepsy

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ABSTRACT

Purpose: Lamotrigine (LTG)-induced maculopapular eruption (MPE) often causes treatment discontinuation and rising burdens on current healthcare systems. We conducted a genome-wide association study to identify novel susceptibility loci associated with LTG-induced MPE in patients with epilepsy.
Materials and methods: We enrolled patients with LTG-induced MPE ($n=34$) and utilized the Korea Association Resource project cohort as a control group ($n=1214$). We explored associations between LTG-induced MPE and single nucleotide polymorphisms (SNPs) through imputation and replicated these associations in samples from 59 LTG-induced MPE cases and 98 LTG tolerant-controls.
Results: We found two novel SNPs associated with LTG-induced MPE: rs12668095 near *CRAMP1L/TMEM204/IFT140/HN1L* ($P=4.89\times10^{-7}$) and rs79007183 near *TNS3* ($P=3.15\times10^{-10}$), both of which were replicated in an independent cohort.
Conclusion: These two validated SNPs may be good candidate markers for predicting LTG-induced MPE in epilepsy patients, although further experimental validation is needed.

1. Introduction

Lamotrigine (LTG) is one of the broad spectrum anti-epileptic drugs (AED) for primary generalized and partial seizure disorder. The most severe adverse effect of LTG is associated with cutaneous adverse drug reactions (cADRs), leading to treatment withdrawal. The incidence of LTG-induced rash is reported between 5% and 10% (Brodie et al., 1995; Matsuo et al., 1993; Messenheimer et al., 1994; Schmidt and Kramer, 1994).

Previous pharmacogenomic studies for AED-induced cADRs focused on carbamazepine (CBZ). These studies showed strong associations between the HLA-B*1502 allele and CBZ-induced Stevens–Johnson syndrome (SJS)/Toxic Epidermal Necrolysis (TEN) in Han Chinese, Thai, Indian, and Malaysian populations (Ding et al., 2010; Locharernkul et al., 2008; Man et al., 2007; Mehta et al., 2009). In European, Japanese, and Korean reports, HLA-A*3101 was associated with cADRs (Kim et al., 2011a,b; McCormack et al., 2011, 2012; Ozeki et al., 2011).

Many previous studies were focused on the gene related to severe cutaneous reactions in LTG, phenytoin etc. However, no genetic marker to significantly predict LTG-induced maculopapular eruption (MPE) has been detected yet (An et al., 2010; Hu et al., 2011; McCormack et al., 2011, 2012). Thus, in the present study, we sought to identify novel susceptibility loci associated with LTG-induced MPE in Korean patients with epilepsy using an integrated genome-wide association study (GWAS).

2. Materials and methods

2.1. Study subjects

One hundred thirty-one subjects receiving AEDs for epilepsy treatment were recruited from Yonsei University College of

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Medicine and Seoul National University College of Medicine. Of 131 subjects, 34 unrelated individuals with LTG-induced MPE were enrolled in our study. Among 34 LTG-induced MPE patients, 14 patients received LTG mono therapy, and the remaining 20 patients received combination therapy. Genotypic data of 1214 individuals from the Korea Association Resource (KARE) project (http://biomi.cdc.go.kr) were used as population control subjects (Cho et al., 2009; Kim et al., 2011a,b). All subjects were of Korean descent. The study protocol complied with ethical guidelines of the 1975 Declaration of Helsinki, and every participant or responsible family members gave their written consent for genetic screening after the study details had been fully explained. This study was approved by the Institutional Review Board of Severance Hospital, Yonsei University College of Medicine, Seoul, Korea (IRB No.: 4-2011-0296).

2.2. Genome-wide association study

For GWAS analysis, samples from 34 patients were genotyped with the GeneChip Human Mapping 500K Array Set (Affymetrix) using the standard protocol recommended by the manufacturer. The 1214 control subjects were genotyped with the Genome-Wide Human SNP array 5.0 (Affymetrix) (Cho et al., 2009; Kim et al., 2011a,b). We merged both data sets using PLINK and performed quality control for all samples and genotyped SNPs according to protocols of previous studies (Anderson et al., 2010; Weale, 2010). To evaluate population stratification, we used HapMap Phase III data from four ethnic populations (European [CEU], African [YRI], Japanese [JPT], and Han Chinese [CHB]) and performed principal component analysis (PCA) using the Smartpca software (EIGENSOFT) (Patterson et al., 2006).

2.3. Genome-wide imputation

We imputed the genotypes of 34 patients and 1080 control subjects passing stringent quality control filters using IMPUTE2 (http://mathgen.stats.ox.ac.uk/impute/impute_v2.html) (Howie et al., 2009). For a reference panel, we used 1000 Genomes Project data released March 2012 (Phase I, version 3) provided by the IMPUTE2 website. We used a combination of four ethnic haplotype panels for reference because combination panels may boost imputation performance (Marchini and Howie, 2010). Reference haplotypes consisted of data from 246 African, 181 American, 286 Asian, and 379 European individuals. In total, 4,893,794 SNPs with an estimated imputation accuracy of >0.9 and minor allele frequency (MAF) of >0.005 were included in the association analysis.

2.4. Replication analysis

The criteria for candidate SNP selection were as follows: (1) $P < 1 \times 10^{-8}$ and no strong linkage disequilibrium (LD) or (2) $P < 1 \times 10^{-6}$ with clustering of nearby SNPs and no strong LD. We selected 12 candidate SNPs from the imputed SNPs, and among them, we further excluded 4 SNPs with a possible epilepsy signal using 131 epileptic subjects and 1214 population controls from KARE. We tested these SNPs using the SNaPshot Multiplex System (Applied Biosystems) for replicating data from 157 Korean individuals (59 LTG-induced MPE cases and 98 LTG-tolerant controls). Among 59 LTG-induced MPE patients, 28 patients received LTG mono therapy and the remaining 31 patients received combination therapy. Finally, seven SNPs were analyzed for replication because one SNP was failed probe assay.

2.5. Functional annotation and cis-eQTL

The Encyclopedia of DNA Elements (ENCODE) project (http://genome.ucsc.edu/ENCODE/) was used for functional annotation of candidate SNPs. For the expression quantitative trait locus (cis-eQTL) analysis, we used quantified data from lymphoblast cell lines in the HapMap3 CHB and JPT database of Genevar (GENe Expression VARiation; http://www.sanger.ac.uk/resources/software/genevar/) (Stranger et al., 2012).

2.6. Statistical analysis

Quality control and association analyses for our imputation and replication data were performed using the PLINK software (http://pngu.mgh.harvard.edu/~purcell/plink/) (Purcell et al., 2007). The quantile–quantile plot and Manhattan plot of imputation data were generated using R version 2.15.2 (http://www.r-project.org/). Regional association plotting was performed using LocusZoom (http://csg.sph.umich.edu/locuszoom/) (Pruim et al., 2010). The validation study was done by analyzing samples for replication and then analyzing combined imputation and replication data. Association analysis of the combined samples was performed by using the Cochran–Mantel–Haenszel test, and the Breslow–Day test was used to evaluate the heterogeneity of odds ratios for the two cohorts (Breslow and Day, 1980).

3. Results

3.1. Genome-wide association study and imputation

The workflow of the present study is shown in Fig. 1. We performed a GWAS with 34 patients and 1214 population control subjects, in which no individuals were excluded based on sex check from calculating mean homozygosity rate across X-chromosome. Individuals with a high missing genotype call rate (≥0.96) and/or extreme heterozygosity (±3 s.d. from the mean) ($n = 129$ controls), related individuals ($n = 7$ controls) or those with a PCA score ±6 s.d. from the mean ($n = 1$ control) (Supplementary Fig. 1) were excluded from subsequent analyses. Quality control criteria for SNPs were as follows: MAF > 0.01 in both cases and controls, genotype call rate ≥0.95, in both cases and controls, and Hardy–Weinberg equilibrium of $P \geq 1 \times 10^{-5}$ in controls. A quantile–quantile plot was useful for SNP verifying quality control by showing the distribution of observed vs. expected P values. The genomic inflation factor was 1.046 and suggested a low inflation possibility of GWAS results from population stratification (Supplementary Fig. 2). After stringent quality control, 335,286 SNPs from the 34 patients and 1080 control subjects were used for imputation.

To investigate the loci that were not covered by the GWAS platform, we imputed GWAS genotypes for 34 case subjects and 1080 control subjects after applying quality control parameters and analyzed the associations of these imputed SNPs. We included 4,893,794 SNPs with an estimated imputation accuracy of >0.9 and MAF of >0.005 for the association analysis (Fig. 2). One hundred seventy-nine SNPs showed $P < 1 \times 10^{-6}$ in allelic association tests of imputed genotype data (Supplementary Table 1). Among them, 12 possible candidate SNPs were selected based on the following criteria: (1) independent loci without strong LD ($r^2 > 0.9$) from SNPs with $P < 1 \times 10^{-8}$ or (2) independent loci without strong LD ($r^2 > 0.9$) from SNPs with $P < 1 \times 10^{-6}$ that clustered with nearby SNPs in allelic association tests of imputed genotype data (Table 1). Because we used population controls instead of LTG-tolerant subjects, we excluded SNPs that showed associations with epilepsy signal. To analyze the epilepsy signal, we used genotypic data of 131 subjects taking AEDs for epilepsy treatment and 1214 population controls from KARE, but after stringent quality control filters were applied, 323,735 SNPs from 130 patients and 1081 population control subjects were used. Of the 12 candidate SNPs, 4 SNPs (rs1178326, rs74912790, rs9596863, and rs17084405) with