Genome-wide association study for adverse reactions of antiepileptic drugs in Korean epilepsy patients

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Department of Medical Science
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Directed by Professor Min Goo Lee

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This certifies that the Doctoral Dissertation of So Won Kim is approved.

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의과대학 졸업 이후 저를 6년간 과학자로 지도해주시고 무한한 은혜를 베풀어 주신 스승님 이민구 교수님께 깊이 감사 드립니다. 대학원 생활 동안 우리 약리학교실을 이끌어주신 교수님들 김경환 교수님, 안영수 교수님, 김동구 교수님, 박경수 교수님, 김철훈 교수님, 김주영 교수님께도 깊은 감사를 올립니다. 또한 바쁘신 와중에도 시간 내주시어 논문 지도를 맡아주신 신경과 이병인 교수님, 김원주 교수님, 소아청소년과 김흥동 교수님, 생리학교실 김정훈 교수님께 고개 숙여 감사 인사 올립니다. 마지막으로 학위 과정 및 전문연구요원 과정 도중 임상약리 분야를 수학할 수 있게 해 주시고 졸업 준비 동안 많은 배려를 해 주신 인제대학교 의과대학 약리학교실 신재국 교수님 감사 드립니다.

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ABSTRACT

Genome-wide association study for adverse reactions of antiepileptic drugs in Korean epilepsy patients

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(Directed by Professor Min Goo Lee)

Skin rash is a well-known adverse drug reaction to antiepileptic drugs (AEDs), and can be caused by AED withdrawal. To date, drug research has focused on carbamazepine (CBZ) in many populations (including Korean), and HLA gene polymorphisms as clinical biomarkers of CBZ-induced skin rash have been identified. Lamotrigine (LTG) is another rash-inducing AED, but biomarkers for LTG-induced skin rash have not been identified. Therefore, 34 epilepsy patients with skin rash derived from LTG were genotyped using the Affymetrix 500k SNP array, and an age-matched healthy Korean population cohort containing 1,214 individuals (which were genotyped using the Affymetrix 5.0 SNP array in 2007) was recruited by the Korean NIH. A total of 33 cases and 1,080 control subjects were analyzed as 1 case subject and 134 control subjects were removed according to the individual and marker quality control (QC) thresholds. Genome-wide

association (GWA) was analyzed using the PLINK software, and imputation analysis was performed at sites not covered by the SNP array chip. Six markers of GWA analyses, which were selected by manual review among sites with p values lower than 10^{-5}, and 23 markers manually selected from the imputation analyses were newly genotyped in the replication cohort of LTG-medicated epilepsy patients (59 with skin rash and 98 without skin rash). Of the 29 selected markers, rs13287547 from GWA analysis and rs12668095, rs17149848, and rs79007183 from imputation analysis were significant (p values of 0.027, 0.009, 0.024, and 0.025 based on allelic analysis, respectively). Rs13287547 and rs12668095 were located at intergenic locations near the C9orf92/BNC2 and TNS3 genes, and rs17149848 and rs79007183 were located at intronic regions within GRM8 and CRAMP1L genes, respectively. This study may facilitate the identification of clinical biomarkers for lamotrigine-induced skin rash, but further investigation is required to determine how these intergenic markers influence the function of nearby genes in different populations, including Koreans.

Key words: Epilepsy, lamotrigine, skin rash, maculopapular eruption, pharmacogenomics, GWA, whole-genome SNP assay

Genome-wide association study for adverse reactions of antiepileptic drugs in Korean epilepsy patients

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I. INTRODUCTION

Lamotrigine (LTG) is one of the most common anti-epileptic drugs (AED) for adult and pediatric epilepsy treatment. Although LTG is highly useful for the treatment of various diseases, such as trigeminal neuralgia and bipolar disorder, as well as seizure disorders, it is associated with cutaneous adverse drug reactions (cADRs) causing treatment withdrawal. The incidence of LTG-induced rash is reported to range from 5 to 10%.[1-5] The incidence of rash induced by LTG is far higher than by any other frequently used AEDs, and LTG is one of the most common rash-inducing AEDs.[6] Of the severe ADRs of LTG, such as rash, pneumonia, and infection, rash is the most common.[7]

To date, previous pharmacogenetic studies for AED-induced cADRs have focused on carbamazepine (CBZ), but not LTG. These studies have revealed a strong association between HLA-B*1502 and CBZ-induced

Stevens-Johnson syndrome (SJS) / toxic epidermal necrolysis (TEN) in Han Chinese, Thai, Indian and Malaysian populations.[8-11] Consequently, the United States Food and Drug Administration have recommended HLA-B*1502 genetic screening before initiating CBZ therapy in Han Chinese and Southeast Asians to protect against SJS/TEN.[12] However, in reports from Europe, Japan and Korea, HLA-A*3101 was associated with CBZ-induced cADRs.[13-15] This finding was based on differences in allelic frequencies of the HLA loci among ethnicities. However, this conclusion—based on HLA gene polymorphisms—is now generally accepted.[16]

Most previous studies of AED-induced cADRs focused on severe cutaneous reactions, such as SJS/TEN and hypersensitivity syndrome (HSS). However, maculopapular eruption (MPE) is more common than SJS/TEN or HSS, and is a burden on the healthcare system. Therefore, pharmacogenetic studies of AED-induced MPE are required.

CBZ-induced MPE is associated with HLA-A*3101 in both European and Han Chinese populations.[14,15,17] In contrast, no known genetic marker is significantly associated with LTG-induced MPE.[18-20] Therefore, we identified novel susceptible loci associated with LTG-induced MPE in Korean populations using GWAS.

II. MATERIALS AND METHODS

1. Study subjects (case subjects /population controls)

Case subjects were recruited from the Neurology Department of Severance Hospital and Seoul National University Hospital (both Seoul, Korea). The case subjects consisted of 34 unrelated individuals with Lamotrigine-induced skin rash. Genotype data from 1,214 individuals from the Korea Association Resource (KARE) project (http://biomi.cdc.go.kr) were used as population control subjects.[21,22] All subjects were of Korean descent. The study protocol complied with the ethical guidelines of the 1975 Declaration of Helsinki. Each participant or the responsible family members gave their consent for genetic screening after the details of the study had been fully explained. The independent Institutional Review Board of our institute approved this study.

2. Genomic DNA preparation

Genomic DNA was prepared from peripheral blood samples using a nucleic acid isolation device, QuickGene-mini80 (FUJIFILM, Tokyo, Japan).

3. GWA genotyping

Genotyping with the Affymetrix Mapping 500K array set (250K Nsp1 and 250K Sty1) was performed at DNALink, using the protocol recommended by the manufacturer. Approximately 250 ng of genomic DNA were digested with two restriction enzymes (*NSP*I and *Sty*I) and processed

according to the Affymetrix protocol.

The digested segments were ligated to enzyme-specific adaptors, which incorporate a universal PCR priming sequence. PCR amplification using universal primers was performed in a reaction optimized to amplify fragments between 200-1,100 base pairs (bp) in length. A fragmentation step then reduced the PCR product to segments of ~25-50 bp, which were end-labeled using biotinylated nucleotides. The labeled product was hybridized to a chip, washed and scanned with the Affymetrix array scanner 30007G. The image was analyzed using the GDAS software (Affymetrix). For the data obtained from each chip, two internal quality-control measures were used: the call rate always exceeded 95%, and heterozygosity on the X chromosome correctly identified the gender of the individual. To confirm that samples were processed correctly, we determined whether the 31 identical SNPs placed on both chips yielded the same genotype for the same individual. Genotype calling was performed using the BRLMM algorithm.

4. SNaPShot genotyping

Genotyping was screened using single-base primer extension assays using ABI PRISM SNaPShot Multiplex kit (ABI, Foster City, CA, USA) according to manufacturer's protocol. Briefly, genomic DNA flanking the SNP of interest was amplified by PCR with forward and reverse primers and standard PCR reagents in a 10-μl reaction volume containing 10 ng of genomic DNA, 0.5 pM of each oligonucleotide primer, 1 μl of 10× PCR buffer, 250 μM dNTP (2.5 mM each) and 0.25 units of DiaStar Taq DNA Polymerase (5 unit/μl) (SolGent co., Ltd. Daejeon, South Korea). The PCR reactions were performed as follows: 10 min at 95°C for 1 cycle, and 35

cycles at 95°C for 30 s, Tm°C for 1 min, and 72°C for 1 min followed by 1 cycle of 72°C for 10 min. After amplification, PCR products were treated with 1 unit of shrimp alkaline phosphatase (SAP) (USB Corporation, Cleveland, OH, USA) and exonuclease I (USB Corporation, Cleveland, OH, USA) at 37°C for 75 min and 72°C for 15 min to purify amplified products. Purified amplification products (1 μl) were added to a SNaPshot Multiplex Ready reaction mixture containing 0.15 pmol of genotyping primer for the primer extension reaction. The primer-extension reaction was performed for 25 cycles at 96°C for 10 s, 50°C for 5 s, and 60°C for 30 s. The reaction products were treated with 1 unit of SAP at 37°C for 1 h and 72°C for 15 min to remove excess fluorescent dye terminators. The final reaction samples (1 μl) containing the extension products were added to 9 μl of Hi-Di formamide (ABI, Foster City, CA). The mixture was incubated at 95°C for 5 min, followed by 5 min on ice and was then analyzed by electrophoresis in an ABI Prism 3730xl DNA analyzer. Analysis was performed using the Genemapper software (version 4.0; Applied Biosystems).

5. SNPtype assay

Genotyping was performed using an SNPtype assay (Fluidigm, San Francisco, CA, USA) according to the manufacturer's protocol. Briefly, the genomic DNA flanking the SNP of interest was amplified using PCR with the STA primer set and Qiagen 2× Multiplex PCR Master Mix (Qiagen) in a 5-μl reaction volume containing 75 ng of genomic DNA. The PCR reactions were performed as follows: 15 min at 95°C for 1 cycle, and 14 cycles at 95°C for 15 s and 60°C for 4 min. After amplification, the STA products

were diluted 1:100 in DNA Suspension Buffer. Diluted STA products (2.5 μl) were added to a Sample Pre-Mix containing 3 μl of 2× Fast Probe Master Mix, 0.3 μl of the SNPtype 20× Sample Loading Reagent, 0.1 μl of the SNPtype Reagent, and 0.036 μl of ROX. After the Assay Pre-Mix and the Sample Pre-Mix were loaded into the 96.96 Dynamic Array, the SNPtype assay reaction was performed as follows: 5 min at 95°C for 1 cycle, 1 cycle at 95°C for 15 s, 64°C for 45 s, and 72°C for 15 s, 1 cycle at 95°C for 15 s, 63°C for 45 s, and 72°C for 15 s, 1 cycle at 95°C for 15 s, 62°C for 45 s, and 72°C for 15 s, 1 cycle at 95°C for 15 s, 61°C for 45 s, and 72° for 15 s, and 34 cycles of 95°C for 15 s, 60°C for 45 s, 72°C for 15 s, and 10 s at 25°C for 1 cycle. Analysis was performed using the Fluidigm SNP Genotyping Analysis software (version 3.1.1; Fluidigm).

6. Genome-wide association study

Samples from 34 cases were genotyped using the GeneChip Human Mapping 500K Array Set (Affymetrix) using the standard protocol recommended by Affymetrix, and 1,214 population control subjects from the KARE project were analyzed using the GeneChip Human Mapping 5.0 Array Set (Affymetrix).

A. Quality control (QC)

Quality control was performed for all samples and SNPs genotyped according to previous studies, as follows.[23,24]

(1) Individual QC

(A) Filter out imprecise sex information

The average homozygosity rate of the X chromosome was calculated for each individual by means of the following command: ‘plink --noweb --bfile raw --check-sex --out raw’, and for output: ‘raw.sexcheck’. FID (family ID, column 1) and IID (individual ID, column 2) of the QC failed samples were organized into ‘fail-sexcheck-qc.txt'.

(B) Identification of individual missingness and heterozygosity

Genotype missingness was evaluated for each individual with the following command: ‘plink --noweb --bfile raw --missing --out raw’, and for output: ‘raw.imiss’ and ‘raw.lmiss’.

The number of heterozygous genotypes without sex chromosomes and nonmissing genotypes per individual were checked with the following command: ‘plink --noweb --bfile raw --het --out raw’, and for output: ‘raw.het’.

‘R CMD BATCH imiss-vs-het.Rscript’, which is provided in the supplementary data of a previous report (http://www.nature.com/nprot/journal/v5/n9/full/nprot.2010.116.html - supplementary-information), was used to visualize heterozygosity rate per individual after installing the geneplotter library of Bioconductor (http://www.bioconductor.org/packages/2.12/bioc/html/geneplotter.html), and output can be obtained at ‘raw.imiss-vs-het.pdf’. FID and IID of QC failed samples were organized into ‘fail-imisshet-qc.txt’.

(C) Filter out duplicated or related individuals

High LD regions ($r^2 > 2$) were excluded from SNP markers before estimating the ancestry relation or duplication with the following command: ‘plink --noweb --bfile raw --exclude high-LD-regions.txt --range --indep-pairwise 50 5 0.2 --out raw’, and markers were written in ‘raw.prune.in’ and ‘raw.prune.out’ output files. ‘High-LD-regions.txt’ are provided in the

supplementary data of a previous report (http://www.nature.com/nprot/journal/v5/n9/full/nprot.2010.116.html - supplementary-information).

IBS (identity by state) was calculated at the remaining markers for all pairs of individuals with the following command: ‘plink --noweb --bfile raw --extract SR-LTG.prune.in --genome --out raw’, and output was written in ‘raw.genome’.

Individuals with IBD (identity by descent) above 0.185 were selected with the following command: ‘perl run-IBD-QC.pl raw’, with the output file: ‘fail-IBD-QC.txt’.

(D) Identification of population stratification

Smartpca from the EIGENSOFT software was used for PCA analysis.[25]

A to T and C to G SNPs were excluded from this analysis with the following command: ‘plink --noweb --bfile raw --extract hapmap3r2_CEU.CHB.JPT.YRI.no-at-cg-snps.txt --make-bed --out raw.hapmap-snps’, and output data: ‘raw.hapmap-snps’ was merged with the HapMap data with the following command: ‘plink --noweb --bfile raw.hapmap-snps --bmerge hapmap3r2_CEU.CHB.JPT.YRI.founders.no-at-cg-snps.bed hapmap3r2_CEU.CHB.JPT.YRI.founders.no-at-cg-snps.bim hapmap3r2_CEU.CHB.JPT.YRI.founders.no-at-cg-snps.fam --extract raw.prune.in --make-bed --out raw.hapmap3r2.pruned’. However, strands had to be flipped with the following command: ‘plink --noweb --bfile raw --flip raw.hapmap3r2.pruned.missnp --extract hapmap3r2_CEU.CHB.JPT.YRI.no-at-cg-snps.txt --make-bed --out raw.hapmap-snps1’ because SNP matching errors occurred. The merging process was performed again with the following command: ‘plink --noweb --bfile raw.hapmap-snps1 --bmerge

hapmap3r2_CEU.CHB.JPT.YRI.founders.no-at-cg-snps.bed hapmap3r2_CEU.CHB.JPT.YRI.founders.no-at-cg-snps.bim hapmap3r2_CEU.CHB.JPT.YRI.founders.no-at-cg-snps.fam --extract raw.prune.in --make-bed --out raw.hapmap3r2.pruned' for the output files: 'SR-LTG.hapmap3r2.pruned.bed/bim/fam'.

'hapmap3r2_CEU.CHB.JPT.YRI.no-at-cg-snps.txt' and 'hapmap3r2_CEU.CHB.JPT.YRI.founders.no-at-cg-snps.bed/bim/fam' files can be obtained from the supplementary data of a previous report (http://www.nature.com/nprot/journal/v5/n9/full/nprot.2010.116.html - supplementary-information).

Prior to PCA analysis, copies of BIM and FAM files were made with different names and the following commands:

'cp raw.hapmap3r2.pruned.bim raw.hapmap3r2.pruned.pedsnp'

'cp raw.hapmap3r2.pruned.fam raw.hapmap3r2.pruned.pedind'

The first PCA analysis was performed using the four ethnic groups CEU, CHB, JPT and YRI, with the following command: 'perl smartpca.perl -i raw.hapmap3r2.pruned.bed -a raw.hapmap3r2.pruned.pedsnp -b raw.hapmap3r2.pruned.pedind -o raw.hapmap3r2.pruned.pca -p raw.hapmap3r2.pruned.plot -e raw.hapmap3r2.pruned.eval -l raw.hapmap3r2.pruned.log -k 2 -t 2 -w pca-populations.txt'. A scatter diagram of PC1 and PC2 can be depicted with the following command: 'plot-pca-results.Rscript', which can be obtained from the supplementary data of a previous report (http://www.nature.com/nprot/journal/v5/n9/full/nprot.2010.116.html - supplementary-information).

FID and IID of QC failed samples were organized into 'fail-ancestry-QC.txt'.

The second PCA analysis using the two ethnic groups CHB and JPT was performed with the same protocols as the first PCA: ‘plink --noweb --bfile raw.hapmap3r2.pruned --keep raw_CHB_JPT.txt --make-bed --out raw.hapmap3r2.ASN.pruned’, after writing the information of CHB and JPT into the new file of ‘raw_CHB_JPT.txt’ from ‘hapmap3r2_CEU.CHB.JPT.YRI.founders.no-at-cg-snps.fam’.

: ‘cp raw.hapmap3r2.ASN.pruned.bim raw.hapmap3r2.ASN.pruned.pedsnp’

: ‘cp raw.hapmap3r2.ASN.pruned.fam raw.hapmap3r2.ASN.pruned.pedind’

: ‘perl smartpca.perl -i raw.hapmap3r2.ASN.pruned.bed -a raw.hapmap3r2.ASN.pruned.pedsnp -b raw.hapmap3r2.ASN.pruned.pedind -o raw.hapmap3r2.ASN.pruned.pca -p raw.hapmap3r2.ASN.pruned.plot -e raw.hapmap3r2.ASN.pruned.eval -l raw.hapmap3r2.ASN.pruned.log -k 2 -t 2 -w pca-populations1.txt’

: ‘plot-pca-results.Rscript’.

FID and IID of QC failed samples were organized into ‘fail-ancestry-QC.txt’.

(E) Removal of all individuals failing QC

Individuals whose measured quality was below the threshold were written together into ‘fail-qc-inds.txt’ with the following command: ‘cat fail-* | sort -k1 | uniq > fail-qc-inds.txt’.

QC failed individuals written into ‘fail-qc-inds.txt’ were removed from the pool with the following command: ‘plink --noweb --bfile raw --remove fail-qc-inds.txt --make-bed --out clean-inds-raw’.

(2) Marker QC

(A) Genotype missing rate calculation

The genotype missing rate of each marker was calculated using the following command: ‘plink --noweb --bfile clean-inds-raw --missing --out

clean-inds-raw', and the output file was created as 'clean-inds-raw.lmiss'.

(B) Comparing call rate between case and control

Genotype call rate of each marker was compared between case and control subjects using the following command: 'plink --noweb --bfile clean-inds-raw --test-missing --out clean-inds-raw', and for the output 'clean-inds-raw.missing'.

SNPs whose p value was lower than 10^{-5} were written into 'fail-diffmiss-qc.txt' with the following command: 'perl run-diffmiss-qc.pl clean-inds-raw'.

(C) Removal of all markers failing QC

All markers failing QC up to the previous steps, as well as MAF and HWE criteria, were removed from the pool using the following command: 'plink --noweb --bfile clean-inds-raw --exclude fail-diffmiss-qc.txt --maf 0.01 --geno 0.05 --hwe 0.00001 --make-bed --out clean-raw' for the output 'clean-raw.bed/bim/fam'.

(D) Removal of all markers failing cluster QC

Cluster QC was performed with markers whose p value was lower than 1×10^{-4} after performing an association test between genotype and phenotype using the following command: 'plink --noweb --bfile clean-raw --assoc --out clean-raw'.

Markers from the failed cluster QC were written in 'fail-cluster-qc.txt' and removed from the pool using the following command: 'plink --noweb --bfile clean-raw --exclude fail-cluster-qc.txt --make-bed --out clean-raw-final', and for the output 'clean-raw-final.bed/bim/fam'.

B. Association test

Individual, marker and cluster QC association tests were performed

using the following command: ‘plink --noweb --bfile clean-raw-final --assoc --out clean-raw-final’.

7. Genome-wide imputation

Genotypes were imputed for 33 case subjects and 1,080 population control subjects with stringent quality control using IMPUTE v. 2.2. For a reference panel, we used the 1000 Genomes Project data released in March 2012 (Phase I, version 3) provided on the IMPUTE2 website. The reference haplotypes consisted of data for 246 African, 181 American, 286 Asian, and 379 European populations. A total of 5,060,642 SNPs with estimated imputation accuracy of greater than 0.9 were included in the association analysis.

8. Replication analysis

A total of 29 candidate SNPs were selected from GWA SNPs and imputed SNPs, and were evaluated using the BioMark HD system and Fluidigm in 157 Korean individuals (59 cases and 98 control subjects).

9. Statistical analysis

All association analyses for our GWAS data were performed using the PLINK software (http://pngu.mgh.harvard.edu/~purcell/plink/) and R software (http://www.r-project.org/) in the Linux operating system.[26] Haploview 4.2 was used for LD mapping of the targeted regions.[27] A regional association plot was generated using LocusZoom.[28]

III. RESULTS

1. Quality control (QC)

Individual QC should precede marker and cluster QC to achieve maximum marker survival.[23] The SNP call rate for all genotyped subjects was ≥ 96%. There was no excluded individual in sexcheck (Figure 1), individual missingness (Figure 2), or duplicated or related individual check using identity-by-descent estimated by PLINK. In the sexcheck procedure, the F-conversed average heterozygosity rate for males was 1 and for females was below 0.2 (Figure 1). One case subject was identified to have large-scale differences in ancestry using PCA and was excluded from the GWA analysis (principle component score ± 6 s.d. from the mean, Figure 3). After exclusion of this subject, all baseline characteristics were not significantly different between the case and control groups (excluding sclerosis ratio). The genomic inflation factor was 1.046 in GWA analysis, suggestive of a low inflation of GWA results from population stratification. 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 PCA using SmartPCA, an *in silico* tool from EIGENSOFT.[25] Based on PCA from four ethnic populations, study subjects included in the JPT and CHB clusters were clearly separated from those in CEU and YRI (Figure 3). A total of seven population control subjects failed the QC of duplicated or related individual check procedures due to IBD values greater than 0.25 (Figure 4). A total of 129 population control subjects more than ± 3 s.d. outside the heterozygosity rate and more than 0.04 for the missing genotype rate also failed the individual missingness and heterozygosity QC procedures (Figure 2).

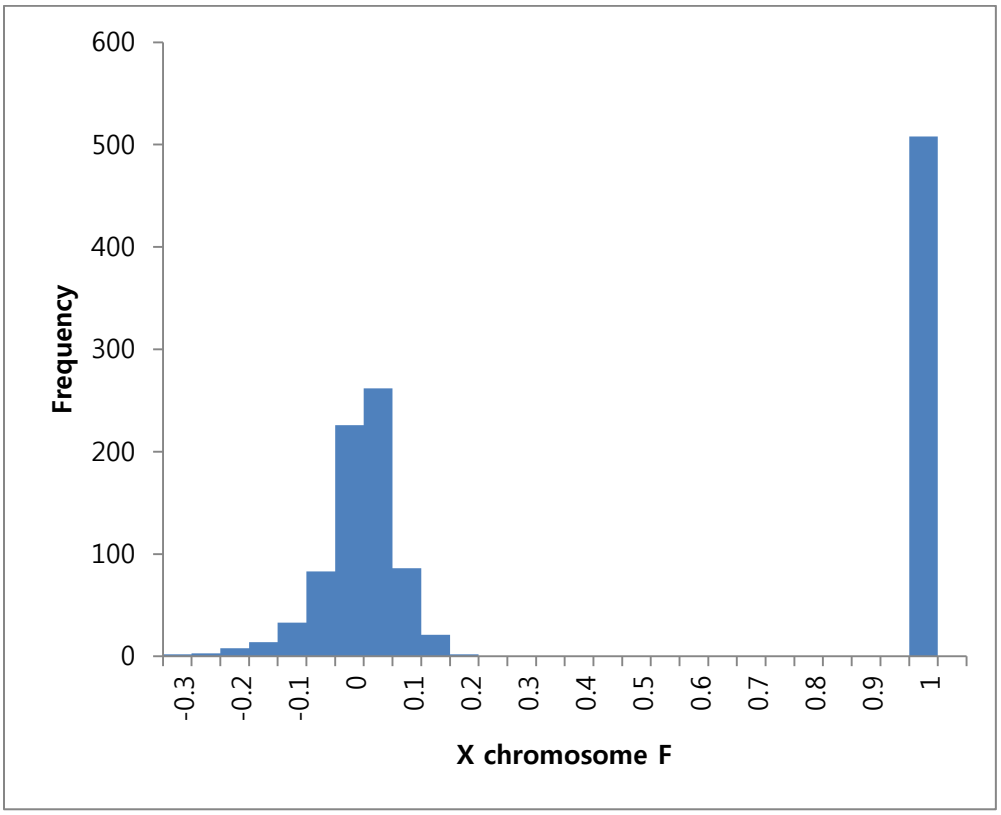

Figure 1. Sex check for quality control

Average heterozygosity of 33 cases and 1,080 control subjects was calculated as the F value, and is depicted on the x-axis. The y-axis indicates the number of subjects.

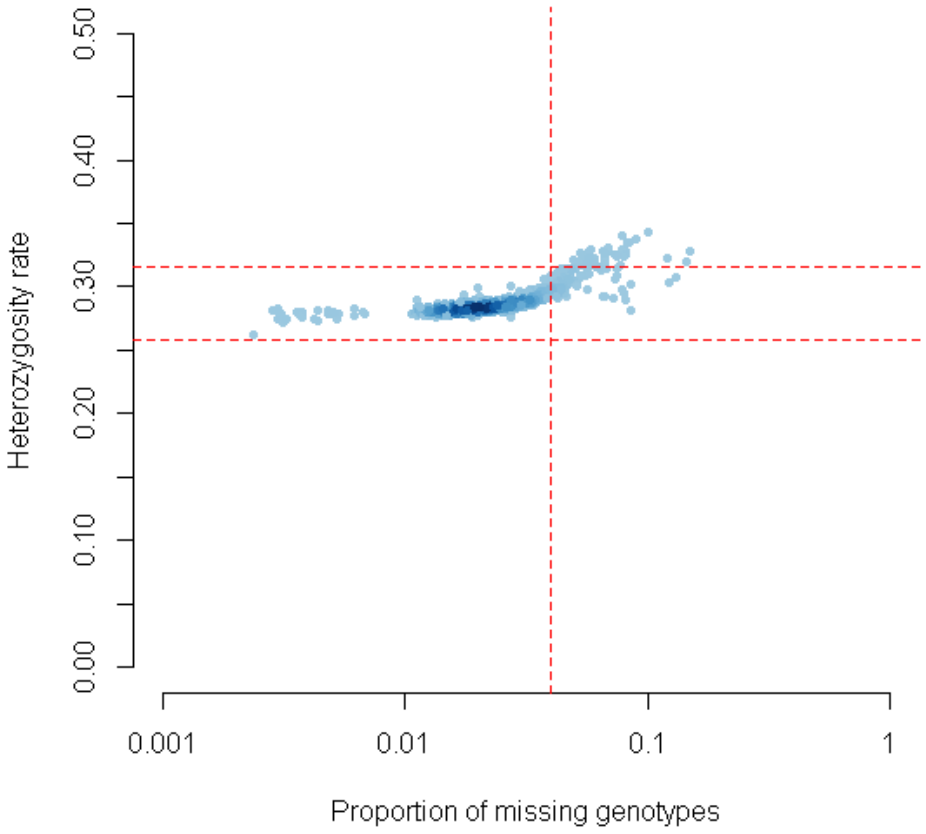

Figure 2. Individual missingness and heterozygosity rate check for quality control

Missing genotype rate (x-axis) and mean heterozygosity rate without the X chromosome (y-axis) per individual are depicted. As the number of overlapping individuals increases, the color darkens. Red dotted line on the x-axis indicates 0.04 and on the y-axis indicates ± 3 standard deviations.

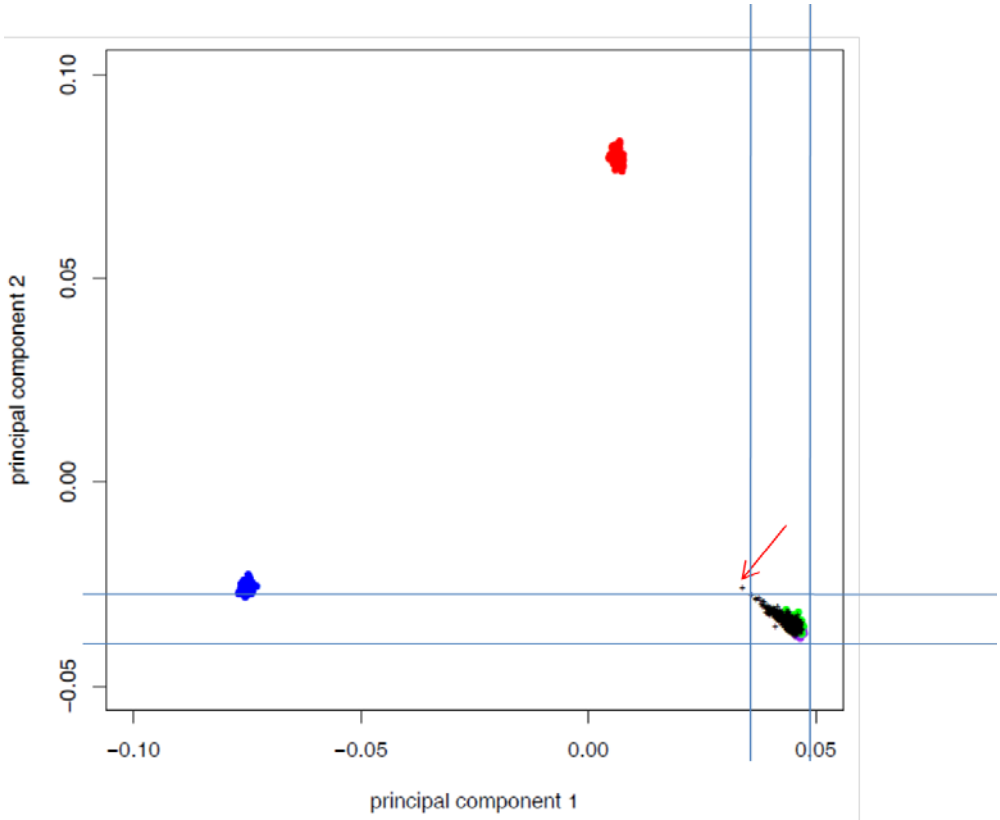

Figure 3. Principal component analysis (PCA) for quality control

Two-dimensional graph of principal components 1 and 2. The four populations and the subjects of this study (34 cases and 1,214 control subjects) are depicted. Vertical and horizontal blue lines indicate ± 6 standard deviations (SD). One individual of 6 SDs of both principal components 1 and 2 is indicated by the red arrow.

Red, CEU; Blue, YRI; Green, CHB, Purple, JPT; Black, subject of this study.

IID1	IID2	Z0	Z1	Z2	PI_HAT	PHE	DST	PPC	RATIO
NIH12B3273128	NIH12B3281598	0.4336	0.5311	0.0353	0.3009	-1	0.86395	1	5.7298
NIH12B3440670	NIH12B3299565	0.4486	0.5514	0	0.2757	-1	0.839731	1	4.8152
NIH12B3063512	NIH12B3138781	0.4492	0.5508	0	0.2754	-1	0.842276	1	5.2957
NIH12B3289629	NIH12B2544780	0.4683	0.5317	0	0.2659	-1	0.857326	1	5.4141
NIH12B3475233	NIH12B2939282	0.4892	0.5066	0.0042	0.2575	-1	0.856736	1	5.4535
NIH12B3255543	NIH12B3126283	0.488	0.512	0	0.256	-1	0.8561	1	5.2598
NIH12B3010594	NIH12B3416483	0.5	0.5	0	0.25	-1	0.853699	1	5.0144
NIH12B2503507	NIH12B3932618	0.5355	0.4305	0.0339	0.2492	-1	0.856886	1	4.7618
NIH12B3274534	NIH12B3519707	0.5016	0.4984	0	0.2492	-1	0.849007	1	4.7616

Figure 4. Duplicated or related individual check for quality control

Seven population-control individuals failed quality control because the PI_HAT values were greater than 0.25.

The threshold for marker QC were a minor allele frequency (MAF) of 0.01 in both cases and control subjects, genotype call rate of 0.95 in both cases and control subjects, and Hardy-Weinberg equilibrium (HWE) p value of 1×10^{-5} in control subjects. As the final procedure, cluster QC was performed for the 1000 top-ranked markers by manual review (Figure 5).

Quantile-quantile plots were depicted for SNP QC by showing the distribution of observed versus expected p values (Figure 6). A range of 0–2 indicated no population stratification or cryptic relatedness, and 4–5 was suggestive of disease association.

After quality control, a total of 335,286 markers with 33 case subjects and 1,080 population control subjects were subjected to analysis (Tables 1, 2).

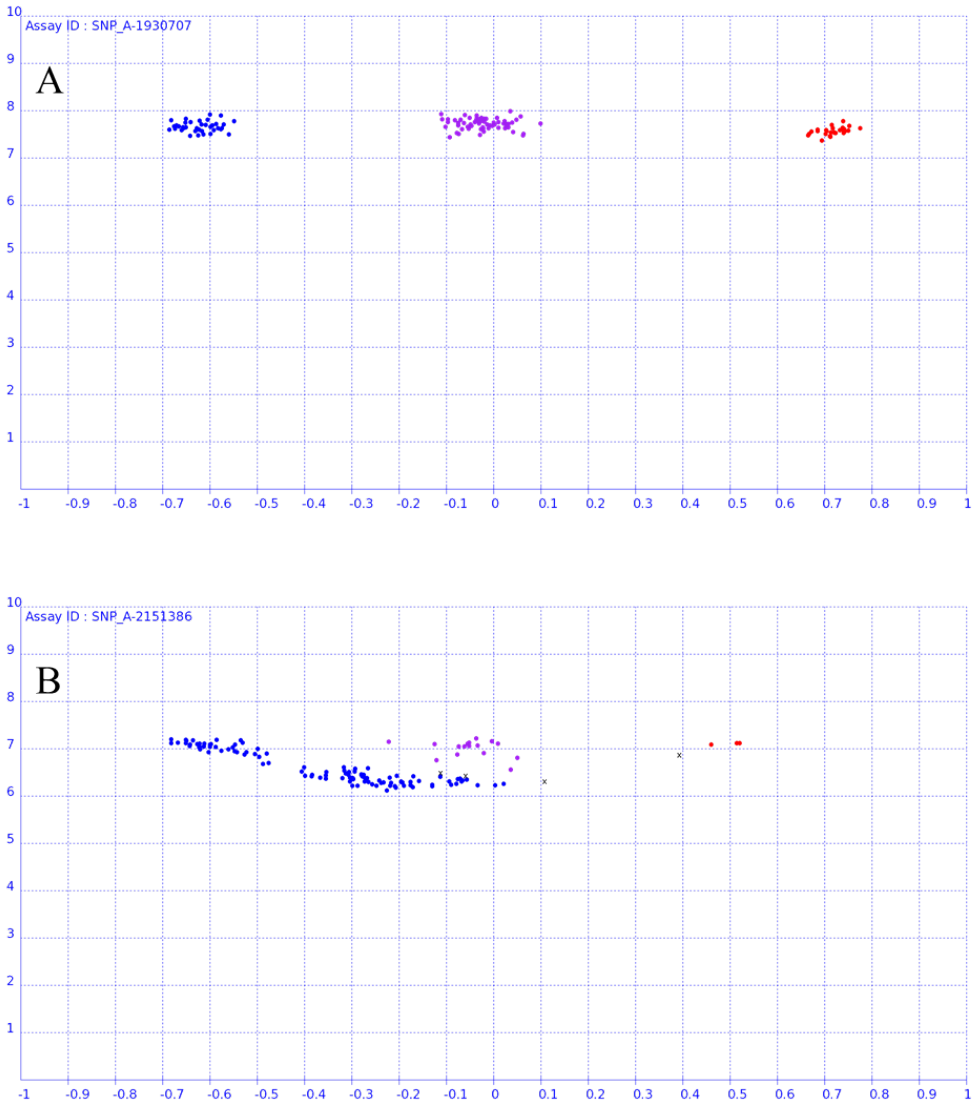

Figure 5. Cluster quality control (QC)

Examples of markers that passed (A, rs300104) and failed (B, 664766) cluster QC.

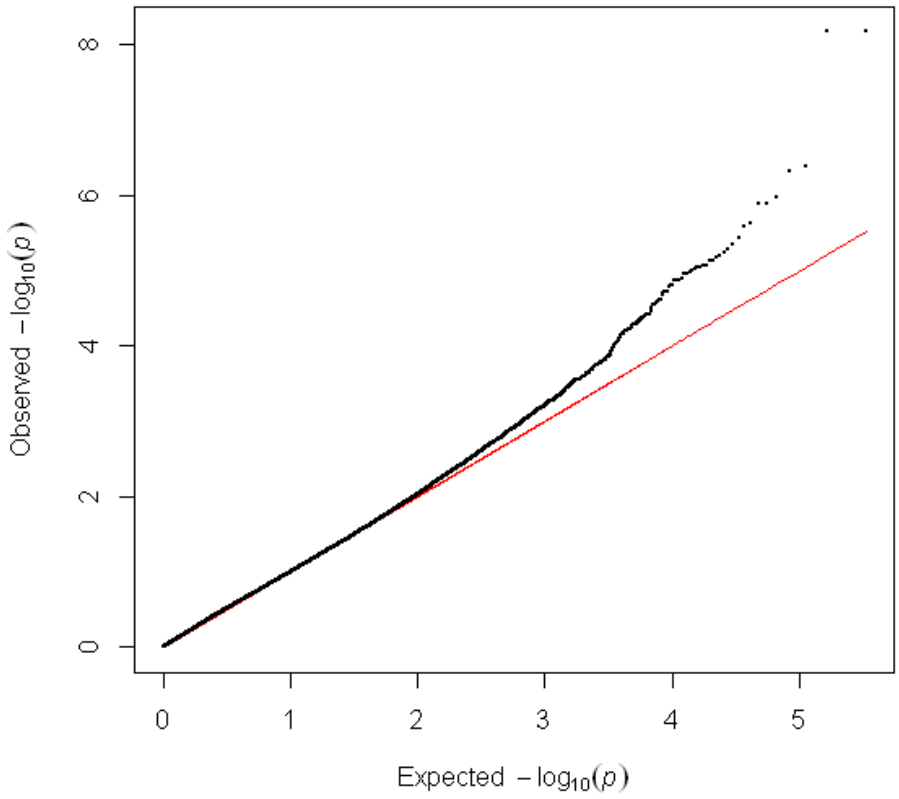

Figure 6. Quantile-quantile (Q-Q) plot

Minus log-scaled expected (x-axis) and observed (y-axis) p values are depicted as black dots. Each black dot indicates one individual of 33 cases and 1,080 controls. Red line indicates that the baseline of expected and observed –log(p)s are equal.

Table 1. Individual and marker comparison before and after quality control (QC).

	Case	Control	Male	Female	Marker
Before QC	34	1,214	508	740	440,398
After QC	33	1,080	461	652	335,286

Table 2. Causes of individual and marker exclusion after quality control.

Individual exclusion
1 case due to population stratification
134 controls
- 129 due to call rate and heterozygosity
- 7 due to related individuals
Marker exclusion
23898 due to missingness test
79080 due to frequency test
7174 due to Hardy-Weinberg equilibrium test
447 due to cluster quality control

2. Genome-wide association study

After stringent quality control, association analysis was completed with 335,733 SNPs of 33 case subjects and 1,080 population control subjects. Six SNPs (rs3796160, rs36164, rs9383054, rs13287547, rs17576443, rs7495694) that represent independent loci were selected according to manual review for replication analysis among 26 SNPs with p values less than 1×10^{-5}. The six selected SNPs comprised three intronic and three intergenic SNPs, and none of the top 26 ranked SNPs were located within coding regions (Figure 7, Table 3).

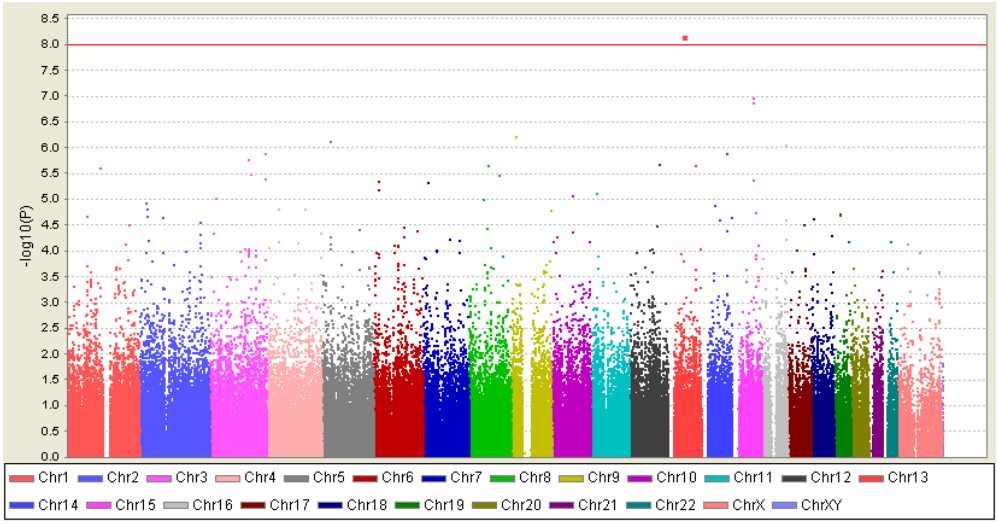

Figure 7. Manhattan plot of GWA analysis.

Chromosomes 1 to 22 and sex chromosomes are lined up along the x-axis; different colors indicate different chromosome positions. The y-axis indicates minus log-scaled p values of GWA analysis.

Table 3. Top-ranked significant SNPs based on GWA analysis

Chr	SNP	Position	A1	A2	χ^2	*p*	OR
13	rs17576443	54153420	G	C	33.56	6.92E-09	9.77
13	rs7335634	54182220	T	C	33.56	6.92E-09	9.77
15	rs7495694	73125992	A	G	28.18	1.11E-07	6.57
15	rs7495960	73126100	T	C	27.76	1.37E-07	6.5
9	rs13287547	16286890	G	A	24.89	6.06E-07	4.84
5	rs6870417	29082654	A	C	24.45	7.61E-07	4.78
5	rs724223	29100354	T	C	24.45	7.61E-07	4.78
16	rs1429264	84623484	C	T	24.14	8.97E-07	8.38
14	rs11628680	88807368	G	A	23.45	1.28E-06	8.07
3	rs3796160	194906614	T	C	23.43	1.30E-06	4.64
3	rs36164	134653602	T	C	22.9	1.70E-06	5.57
12	rs7979041	102201616	A	G	22.44	2.16E-06	3.68
8	rs16932415	66928053	A	G	22.42	2.19E-06	6.02
13	rs4575403	93430257	T	C	22.36	2.27E-06	4.02
1	rs269125	112611387	C	T	22.14	2.53E-06	7.72
3	rs13317029	142961223	G	A	21.64	3.30E-06	4.4
8	rs17790247	104079048	A	G	21.51	3.51E-06	4.01
3	rs1961324	194903701	T	C	21.27	3.99E-06	4.35
15	rs7178889	73111717	T	C	21.16	4.22E-06	5.25
6	rs9383054	15397635	A	G	21.05	4.47E-06	3.19
7	rs1178330	18261466	T	C	20.93	4.78E-06	6.38
6	rs2237156	15391208	G	A	20.31	6.59E-06	3.13
11	rs7939710	20512214	T	A	19.95	7.94E-06	2.89
10	rs17528058	72424452	T	C	19.82	8.50E-06	5.47
3	rs17810253	20766934	G	T	19.6	9.57E-06	4.96
8	rs16914081	50865230	A	G	19.51	9.99E-06	4.6

3. Genome-wide imputation

To identify additional uncovered susceptible loci, genotypes not included within GWA were imputed based on the haplotypes in the 1000 Genome Project data, composed of Asian, African, American, and European populations, for 33 case and 1,080 population control subjects using IMPUTE v. 2.2.

A total of 5,060,642 SNPs with an estimated imputation accuracy of greater than 0.9 were included in the association analysis. A total of 23 manually reviewed SNPs were selected (rs58258683, rs10198754, rs2602797, rs188453989, rs13128807, rs631844, rs71568191, rs12668095, rs1178326, rs17149848, rs116881852, rs150435906, rs181734534, rs74308953, rs17528058, rs1147013, rs7112588, rs9596863, rs118166657, rs79007183, rs62103681, rs1983974, and rs404148) to represent independent loci among 441 SNPs with p values less than 1×10^{-5}, which included 353 SNPs in allelic association tests and 88 SNPs in recessive association tests (Figure 8).

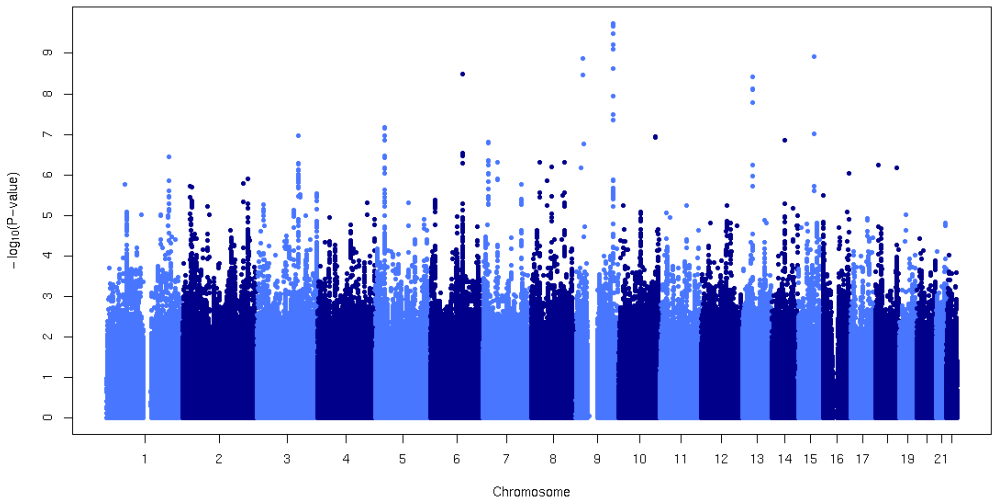

Figure 8. Manhattan plot of imputation analysis

Chromosomes 1 to 22 and sex chromosomes are lined up along the x-axis according to their position on the chromosome. The y-axis indicates minus log-scaled p values by GWA analysis.

4. Replication

A total of 29 SNPs from 6 GWA analyses and 23 imputation analyses were selected for replication-association analysis. For the replication cohort, 59 LTG-medicated Korean epilepsy patients with skin rash and 98 without skin rash were newly recruited at the Severance Hospital Department of Neurology. Of the 29 SNPs, rs13287547 from GWA analysis and rs12668095, rs17149848, and rs79007183 from imputation analysis showed significance in the allelic analysis (p values of 0.027, 0.009, 0.024, and 0.025 respectively, Table 4).

Table 4. Association analysis of the replication cohort.

	SNP	Chr	Position	Nearest Gene	Locus	Discovery *p*	Replication *p*	Epilepsy *p*
GWA	rs36164	3	134653602	EPHB1	intron	1.70E-06	0.9849	9.25E-05
	rs3796160	3	194906614	XXYLT1	intron	1.30E-06	0.0934	0.0764
	rs9383054	6	15397635	JARID2	intron	4.47E-06	0.5826	0.019
	rs13287547	9	16286890	BNC2	intergenic	6.06E-07	0.0268	0.0044
	rs17576443	13	54153420	LINC00558	intergenic	6.92E-09	0.4133	0.0001
	rs7495694	15	73125992	ADPGK-AS1	intergenic	1.11E-07	0.2586	0.0736
Imputation	rs58258683	1	201080785	CACNA1S	intron	9.95E-06	0.6057	0.0647
	rs10198754	2	212440041	ERBB4	intron	1.22E-06	0.3674	0.0015
	rs2602797	2	30859968	LCLAT1	Intron	2.00E-06	0.385	0.0125
	rs188453989	3	42703412	ZBTB47	Intron	3.65E-06	0.1968	0.0112
	rs13128807	4	170232880	SH3RF1	Intron	1.83E-06	0.4371	6.65E-05
	rs631844	5	29054620	none	Intergenic	6.59E-08	0.3589	0.0113
	rs71568191	6	104678832	HACE1	Intergenic	3.29E-09	0.9962	0.1571
	rs1178326	7	18234857	HDAC9	Intron	1.55E-07	0.6057	7.26E-05
	rs12668095	7	47075776	TNS3	Intergenic	4.89E-07	0.0094	0.0013
	rs17149848	7	126133668	GRM8	Intron	1.68E-06	0.0238	0.0018
	rs116881852	8	48351748	KIAA0146	Intron	5.77E-08	0	0.0016
	rs150435906	8	66864338	DNAJC5B/PDE7A	Intergenic	6.31E-07	0	0.0036
	rs181734534	8	52093344	PXDNL	Intergenic	1.93E-10	0	0.0715
	rs74308953	9	121431189	none	Intergenic	1.79E-10	0.3331	0.2278

rs17528058	10	72424452	ADAMTS14	Intergenic	9.00E-06	0.2586	0.0083
rs1147013	11	104576373	CASP12	Intergenic	5.77E-08	0	0.0103
rs7112588	11	104168369	PDGFD	Intergenic	1.88E-13	0	7.82E-06
rs9596863	13	54434647	LINC00558	Intron	8.02E-11	0.6944	4.84E-05
rs118166657	14	64047262	WDR89	Intergenic	1.40E-07	0.7656	0.0091
rs79007183	16	1705794	CRAMP1L	Intron	3.15E-10	0.0249	0.0604
rs62103681	18	69123729	LOC100505776	Intergenic	7.89E-09	0.4133	0.0002
rs1983974	20	25530619	NINL	Intron	1.81E-06	0.4371	0.0018
rs404148	20	25447931	NINL	Intron	5.77E-08	0.4371	0.0003

IV. DISCUSSION

Of the Korean cohort analyzed using an Affymetrix SNP chip 5.0 including 8,842 individuals recruited by the Korean National Institute of Health in 2007 (KARE data), 1,214 healthy subjects with baseline characteristics that matched case subjects were used as a population control. Using a large population control in a genome-wide association study increases the statistical power compared to using clinical controls and a small number of subjects.[29] However, association analysis with population control makes it difficult to identify markers associated with epilepsy or lamotrigine-induced skin rash. To address this issue, clinical control was performed using lamotrigine-treated epilepsy patients without skin rash as a replication cohort. Not all markers from the association results maintained significance in the replication analysis because the two control groups used in this study had different characteristics. This strategy of using population control during the first association and clinical control during the replication analysis can reduce the type I error rate. Since the same manufacturer and similar versions of genotyping SNP assay flatforms were used for case and control subjects, the probability of type I error may be reduced when flatforms from different manufacturers are used.[29] The misidentification of skin rash and epilepsy can be avoided based on both theoretical assumptions and by confirming signals for epilepsy during the GWA and imputation analysis phase. Compared with the skin rash signal, *p* values for epilepsy were lower and did not exceed the cutoff value of 10^{-5}. The *p* values for epilepsy signals of rs13287547, rs12668095, rs17149848, and rs79007183 were 0.004, 0.001, 0.002, and 0.06, respectively.

Imputation analysis was applied to address deficits in the GWAS SNP assay or was combined with different SNP assay flatforms, similar to

recent GWAS. For accurate prediction based on a case group, LD-based prediction is required for a large population with similar characteristics, such as epilepsy with skin rash due to lamotrigine. However, it is difficult to recruit a large population cohort with specific conditions; indeed, no such cohort was available in Korea. Thus, we used the full sequencing data of 1000 Genome Projects, which likely increases type I error due to inaccurate prediction, because imputation is based on a statistical technique. Therefore, the degree of reliability during genotyping analysis may be improved compared to imputation.[29]

Previous reports focused mainly on carbamazepine for AED-induced skin rash in many populations (including Koreans), and was shown to be associated with HLA gene polymorphisms such as HLA-A*3101 and HLA-B*1502.[16] However, lamotrigine-induced skin rash in this study did not show any relationship with HLA genes. This may be due to differences in AED characteristics. Although skin rash caused by AEDs is classified as type B (idiosyncratic), which is not dose related, lamotrigine-induced skin rash is thought to be correlated with the initial dose or dose titration rate.[30] We were not able to analyze the influence of dose because the dose that induced rash and the dose elevation degree until developing a rash varied among patients, and there the number of case subjects was insufficient to divide into groups according to dose. Different mechanisms of action may also affect the outcome of these drugs. For example, although both lamotrigine and carbamazepine are sodium channel blockers, they have opposite effects on GABA, and lamotrigine also inhibits glutamate release. Whether these mechanisms are associated with skin rash remains unclear.[31-34]

Of the four significant SNPs in the first and replication cohorts, rs13287547 and rs12668095 were located in intergenic sequences. Two

genes, C9orf92 and BNC2, were located close to rs13287547. Although the function of C9orf92 is not well understood, a putative similar gene, C9orf72 located on the same chromosome (chromosome 9), is known to be associated with several diseases, such as familial amyotrophic lateral sclerosis (ALS),[35] frontotemporal dementia[36] and Parkinsonism.[37,38] However, recent reports suggested that C9orf72 repeat expansion is not likely the main cause of familial and sporadic ALS in Koreans.[39] Likewise, it remains unclear whether mutation in C9orf72 is a primary cause of Parkinsonism in many populations.[40-43] BNC2, another gene in close proximity to rs13287547, is known to be associated with ovarian development[44] and cancer.[45,46] Lastly, the TNS3 gene near rs12668095 is known to be related to thyroid and kidney cancer.[47,48] However, these two polymorphisms and three genes may not cause the phenotype observed in this study. These two SNPs may influence the three genes mentioned above, as well as others yet to be identified. Recently, the usefulness of intergenic sequences has become apparent. Not only gene regulatory elements, such as promoters and enhancers, but also non-natural protein were artificially synthesized from intergenic sequences.[49]

The remaining two SNPs (rs17149848 and rs79007183) were located at intronic regions within GRM8 and CRAMP1L, respectively. Although not all association results of previous studies were significant, several groups have reported a relationship between polymorphisms of GRM8, which encodes a glutamate receptor, with psychiatric phenomena such as schizophrenia,[50] autistic disorder,[51] panic disorder,[52] heroin addiction,[53] and alcohol dependence.[54] The function of CRAMP1L was previously unknown. Therefore, characterizing these markers in Koreans and further research into the underlying mechanism is required to identify applicable clinical markers.

This study is the first to identify a genetic biomarker for lamotrigine-induced skin rash in Korean epilepsy patients. rs13287547, rs12668095, rs17149848 and rs79007183 may facilitate prediction of lamotrigine-induced skin rash since they were confirmed in the replication analysis in this study. However, further studies are required to identify suitable clinical markers.

V. CONCLUSION

In this study, 34 Korean epilepsy patients with lamotrigine-induced skin rash and a Korean population cohort comprising 1,214 individuals was genotyped using Affymetrix whole-genome SNP array. After quality control, 33 cases, 1,080 controls, and 335,286 markers were analyzed, and more markers were included in the analysis using *in silico* imputation techniques. Six SNPs from the GWA analysis and 23 SNPs from the imputation analysis were selected for replication analysis by manual review based on their top-ranked significant p values.

The replication cohort of 59 lamotrigine-medicated Korean epilepsy patients with skin rash, and 98 lamotrigine-medicated Korean epilepsy patients without skin rash, was newly recruited. Of the 29 SNPs, rs13287547 from GWA analysis and rs12668095, rs17149848, rs79007183 from imputation analysis showed significant p values in the allelic association test (0.027, 0.009, 0.024 and 0.025, respectively). These four SNPs are located in the intergenic regions near the genes C9orf92/BNC2 and TNS3, and intronic regions within GRM8 and CRAMP1L genes, respectively.

This study is the first to identify genetic markers associated with lamotrigine-induced skin rash in Korean epilepsy patients, and can be used for future practical clinical biomarker discovery.

REFERENCES

1. Matsuo F, Bergen D, Faught E, Messenheimer JA, Dren AT, Rudd GD, et al. Placebo-controlled study of the efficacy and safety of lamotrigine in patients with partial seizures. U.S. Lamotrigine Protocol 0.5 Clinical Trial Group. Neurology 1993;43:2284-91.
2. Messenheimer J, Ramsay RE, Willmore LJ, Leroy RF, Zielinski JJ, Mattson R, et al. Lamotrigine therapy for partial seizures: a multicenter, placebo-controlled, double-blind, cross-over trial. Epilepsia 1994;35:113-21.
3. Schmidt D, Kramer G. The new anticonvulsant drugs. Implications for avoidance of adverse effects. Drug Saf 1994;11:422-31.
4. Brodie MJ, Richens A, Yuen AW. Double-blind comparison of lamotrigine and carbamazepine in newly diagnosed epilepsy. UK Lamotrigine/Carbamazepine Monotherapy Trial Group. Lancet 1995;345:476-9.
5. Medicines CoSo. Reminder: Lamotrigine (Lamictal) and serious skin reactions. Current Problem in Pharmacovigilance 1996;22:11.
6. Arif H, Buchsbaum R, Weintraub D, Koyfman S, Salas-Humara C, Bazil CW, et al. Comparison and predictors of rash associated with 15 antiepileptic drugs. Neurology 2007;68:1701-9.
7. Valencia I, Pinol-Ripoll G, Khurana DS, Hardison HH, Kothare SV, Melvin JJ, et al. Efficacy and safety of lamotrigine monotherapy in children and adolescents with epilepsy. Eur J Paediatr Neurol 2009;13:141-5.
8. Man CB, Kwan P, Baum L, Yu E, Lau KM, Cheng AS, et al. Association between HLA-B*1502 allele and antiepileptic drug-induced cutaneous reactions in Han Chinese. Epilepsia 2007;48:1015-8.

9. Locharernkul C, Loplumlert J, Limotai C, Korkij W, Desudchit T, Tongkobpetch S, et al. Carbamazepine and phenytoin induced Stevens-Johnson syndrome is associated with HLA-B*1502 allele in Thai population. Epilepsia 2008;49:2087-91.
10. Mehta TY, Prajapati LM, Mittal B, Joshi CG, Sheth JJ, Patel DB, et al. Association of HLA-B*1502 allele and carbamazepine-induced Stevens-Johnson syndrome among Indians. Indian J Dermatol Venereol Leprol 2009;75:579-82.
11. Ding WY, Lee CK, Choon SE. Cutaneous adverse drug reactions seen in a tertiary hospital in Johor, Malaysia. Int J Dermatol 2010;49:834-41.
12. Ferrell PB, Jr., McLeod HL. Carbamazepine, HLA-B*1502 and risk of Stevens-Johnson syndrome and toxic epidermal necrolysis: US FDA recommendations. Pharmacogenomics 2008;9:1543-6.
13. Kim SH, Lee KW, Song WJ, Kim SH, Jee YK, Lee SM, et al. Carbamazepine-induced severe cutaneous adverse reactions and HLA genotypes in Koreans. Epilepsy Res 2011;97:190-7.
14. McCormack M, Alfirevic A, Bourgeois S, Farrell JJ, Kasperaviciute D, Carrington M, et al. HLA-A*3101 and carbamazepine-induced hypersensitivity reactions in Europeans. N Engl J Med 2011;364:1134-43.
15. Ozeki T, Mushiroda T, Yowang A, Takahashi A, Kubo M, Shirakata Y, et al. Genome-wide association study identifies HLA-A*3101 allele as a genetic risk factor for carbamazepine-induced cutaneous adverse drug reactions in Japanese population. Hum Mol Genet 2011;20:1034-41.
16. Amstutz U, Ross CJD, Castro-Pastrana, LI, Rieder MJ, Shear NH, Hayden MR, et al. HLA-A*31:01 and HLA-B*15:02 as genetic markers for carbamazepine hypersensitivity in children. Clin Pharmacol Ther

2013;94:142-9.

17. Hung SI, Chung WH, Jee SH, Chen WC, Chang YT, Lee WR, et al. Genetic susceptibility to carbamazepine-induced cutaneous adverse drug reactions. Pharmacogenet Genomics 2006;16:297-306.
18. An DM, Wu XT, Hu FY, Yan B, Stefan H, Zhou D. Association study of lamotrigine-induced cutaneous adverse reactions and HLA-B*1502 in a Han Chinese population. Epilepsy Res 2010;92:226-30.
19. Hu FY, Wu XT, An DM, Yan B, Stefan H, Zhou D. Pilot association study of oxcarbazepine-induced mild cutaneous adverse reactions with HLA-B*1502 allele in Chinese Han population. Seizure 2011;20:160-2.
20. McCormack M, Urban TJ, Shianna KV, Walley N, Pandolfo M, Depondt C, et al. Genome-wide mapping for clinically relevant predictors of lamotrigine- and phenytoin-induced hypersensitivity reactions. Pharmacogenomics 2012;13:399-405.
21. Cho YS, Go MJ, Kim YJ, Heo JY, Oh JH, Ban HJ, et al. A large-scale genome-wide association study of Asian populations uncovers genetic factors influencing eight quantitative traits. Nat Genet 2009;41:527-34.
22. Kim YJ, Go MJ, Hu C, Hong CB, Kim YK, Lee JY, et al. Large-scale genome-wide association studies in East Asians identify new genetic loci influencing metabolic traits. Nat Genet 2011;43:990-5.
23. Anderson CA, Pettersson FH, Clarke GM, Cardon LR, Morris AP, Zondervan KT. Data quality control in genetic case-control association studies. Nat Protoc 2010;5:1564-73.
24. Weale ME. Quality control for genome-wide association studies. Methods Mol Biol 2010;628:341-72.
25. Patterson N, Price AL, Reich D. Population structure and eigenanalysis. PLoS Genet 2006;2:e190.

26. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559-75.
27. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263-5.
28. Pruim RJ, Welch RP, Sanna S, Teslovich TM, Chines PS, Gliedt TP, et al. LocusZoom: regional visualization of genome-wide association scan results. Bioinformatics 2010;26:2336-7.
29. Tian C, Gregersen PK, Seldin MF. Accounting for ancestry: population substructure and genome-wide association studies. Hum Mol Genet 2008;17:R143-50.
30. Perucca E, Meador KJ. Adverse effects of antiepileptic drugs. Acta Neurol Scand Suppl 2005;181:30-5.
31. Thomas SP, Nandhra HS, Jayaraman A. Systematic review of lamotrigine augmentation of treatment resistant unipolar depression (TRD). J Ment Health 2010;19:168-75.
32. Ketter TA, Manji HK, Post RM. Potential mechanisms of action of lamotrigine in the treatment of bipolar disoders. J Clin psychopharmacol 2003;23:484-95.
33. Braga MF, Aroniadou-Anderjaska V, Post RM, Li H. Lamotrigine reduces spontaneous and evoked GABAA receptor-mediated synaptic transmission in the basolateral amygdala: implications for its effects in seizure and affective disorders. Neuropharmacology 2002;42:522-9.
34. Shiah IS, Yatham LN, Gau YC, Baker GB. Effect of lamotrigine on plasma GABA levels in healthy humans. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:419-23.
35. García-Redondo A, Dols-Icardo O, Rojas-García R, Esteban-Pérez

J, Cordero-Vázquez P, Muñoz-Blanco JL, et al. Analysis of the C9orf72 gene in patients with amyotrophic lateral sclerosis in Spain and different populations worldwide. Hum Mutat 2013;34:79-82.

36. Harms M, Benitez BA, Cairns N, Cooper B, Cooper P, Mayo K, et al. C9orf72 hexanucleotide repeat expansions in clinical Alzheimer disease. JAMA Neurol 2013;70:736-41.
37. Lesage S, Le Ber I, Condroyer C, Broussolle E, Gabelle A, Thobois S, et al. C9orf72 repeat expansions are a rare genetic cause of parkinsonism. Brain 2013;136:385-91.
38. O'Dowd S, Curtin D, Waite AJ, Roberts K, Pender N, Reid V, et al. C9orf72 expansion in amyotrophic lateral sclerosis/frontotemporal dementia also causes parkinsonism. Mov Disord 2012;27:1072-4.
39. Jang JH, Kwon MJ, Choi WJ, Oh KW, Koh SH, Ki CS, et al. Analysis of the C9orf72 hexanucleotide repeat expansion in Korean patients with familial and sporadic amyotrophic lateral sclerosis. Neurobiol Aging 2013;34:1131.e7-9.
40. Kohli MA, John-Williams K, Rajbhandary R, Naj A, Whitehead P, Hamilton K, et al. Repeat expansions in the C9orf72 gene contribute to Alzheimer's disease in Caucasians. Neurobiol Aging 2013;34:1519.e5-12.
41. Dejesus-Hernandez M, Rayaprolu S, Soto-Ortolaza AI, Rutherford NJ, Heckman MG, Traynor S, et al. Analysis of the C9orf72 repeat in Parkinson's disease, essential tremor and restless les syndrome. Parkinsonism Relat Disord 2013;19:198-201.
42. Akimoto C, Forsgren L, Linder J, Birve A, Backlund I, Andersson J, et al. No GGGGCC-hexanucleotide repeat expansion in C9ORF72 in parkinsonism patients in Sweden. Amyotroph Lateral Scler

Frontotemporal Degener 2013;14:26-9.

43. Majounie E, Abramzon Y, Renton AE, Keller MF, Traynor BJ, Singleton AB. Large C9orf72 repeat expansions are not a common cause of Parkinson's disease. Neurobiol Aging 2012;33:2527.e1-2.
44. Wentzensen N, Black A, Jacobs K, Yang HP, Berg CD, Caporaso N, et al. Genetic variation on 9p22 is associated with abnormal ovarian ultrasound results in the prostate, lung, colorectal, and ovarian cancer screening trial. PLoS One 2011;6:e21731.
45. Sundqvist J, Falconer H, Seddighzadeh M, Vodolazkaia A, Fassbender A, Kyama C, et al. Ovarian cancer-associated polymorphisms in the BNC2 gene among women with endometriosis. Hum Reprod 2011;26:2253-7.
46. Goode EL, Chenevix-Trench G, Song H, Ramus SJ, Notaridou M, Lawrenson K, et al. A genome-wide association study identifies susceptibility loci for ovarian cancer at 2q31 and 8q24. Nat Genet 2010;42:874-9.
47. Maeda I, Yamada H, Takano T, Nishihara E, Ito Y, Matsuzuka F, et al. Increased expression levels of tensin3 mRNA in thyroid functional adenomas as compared to non-functioning adenomas. Exp Clin Endocrinol Diabetes 2009;117:191-3.
48. Martuszewska D, Ljungberg B, Johansson M, Landberg G, Oslakovic C, Dahlbäck B, et al. Tensin3 is a negative regulator of cell migration and all four Tensin family members are downregulated in human kidney cancer. PloS One 2009;4:e4350.
49. Dhar PK, Thwin CS, Tun K, Tsumoto Y, Maurer-Stroh S, Eisenhaber F, et al. Synthesizing non-natural parts from natural genomic template. J Biol Eng 2009;3:3:2.

50. Takaki H, Kikuta R, Shibata H, Ninomiya H, Tashiro N, Fukumaki Y. Positive associations of polymorphisms in the metabotropic glutamate receptor type 8 gene (GRM8) with schizophrenia. Am J Med Genet B Neuropsychiatr Genet 2004;128B:6-14.
51. Li H, Li Y, Shao J, Li R, Qin Y, Xie C, et al. The association analysis of RELN and GRM8 genes with autistic spectrum disorder in Chinese Han population. Am J Med Genet B Neuropsychiatr Genet 2008;147B:194-200.
52. Kobayashi Y, Akiyoshi J, Kanehisa M, Ichioka S, Tanaka Y, Tsuru J, et al. Lack of polymorphism in genes encoding mGluR 7, mGluR 8, GABA(A) receptor alfa-6 subunit and nociceptin/orphanin FQ receptor and panic disorder. Psychiatr Genet 2007;17:9.
53. Nielsen DA, Ji F, Yuferov V, Ho A, Chen A, Levran O, et al. Genotype patterns that contribute to increased risk for or protection from developing heroin addiction. Mol Psychiatry 2008;13:417-28.
54. Chen AC, Tang Y, Rangaswamy M, Wang JC, Almasy L, Foroud T, et al. Association of single nucleotide polymorphisms in a glutamate receptor gene (GRM8) with theta power of event-related oscillations and alcohol dependence. Am J Med Genet B Neuropsychiatr Genet 2009;150B:359-68.

ABSTRACT (IN KOREAN)

한국인 간질 환자에서 항간질제의 부작용의 원인에 대한 전장 유전체 연관 연구

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김소원

피부 발진(skin rash)은 항간질약제(antiepileptic drug)의 대표적인 부작용 중 하나로서 약의 중단의 원인이 되는 심각한 부작용이다. 지금까지 카바마제핀(carbamazepine)에 의한 피부 발진의 원인 발굴 연구가 한국인을 포함한 여러 민족에서 진행되었고, HLA 유전자의 염기 다형성들이 카바마제핀 유발 피부 발진의 임상적 바이오마커로 발굴되었다. 라모트리진(lamotrigine) 또한 피부 발진을 유발하는 대표적인 항간질약제의 하나이며 아직까지 바이오마커 연구가 이루어지지 않았다. 따라서 라모트리진으로 피부 발진이 유발된 간질 환자 34명의 유전형을 Affymetrix 500k SNP array로 분석하고 한국 국립보건연구원에서 2007년에 Affymetrix 5.0 SNP array로 분석한 건강한 한국인 코호트 1214명의 유전형을 분양받았다. 개인별, 마커별 품질

관리(quality control)에서 실험군 1명, 대조군 134명이 탈락하여 최종 실험군 33명과 대조군 1080명으로 분석하였다. PLINK 프로그램으로 전장 유전체 연관 분석을 진행하였고, SNP array로 커버 불가능한 지역은 imputation으로 분석하였다. 전장 유전체 연관 분석에서 10^{-5}보다 낮은 p 값을 가진 마커 중 검토를 통해 선정한 6개의 마커와, imputation 분석에서 검토를 통해 선정된 23개의 마커를 사용하여 라모트리진을 복용한 간질 환자 코호트를 새로 모집한 후 59명의 피부 발진 발생자와 98명의 비 발생자의 유전형을 검사하였다. 총 29개의 마커 중 GWA 분석 마커인 rs13287547과 imputation 분석 마커인 rs12668095, rs17149848, rs79007183이 대립 유전자 분석에서 각각 0.027, 0.009, 0.024 및 0.025의 유의한 p값을 나타내었다. rs13287547과 rs12668095는 각각 C9orf92/BNC2와 TNS3 유전자 근처의 비유전자 지역에, 그리고 rs17149848과 rs79007183은 GRM8과 CRAMP1L의 유전자 내부 인트론 지역에 위치한다. 한국인을 포함한 여러 민족에서 이 마커들의 유의성 확인을 위한 연구 및 주변 유전자의 기능에 미치는 영향을 탐색하는 연구가 추가적으로 필요하며, 이 연구가 라모트리진으로 인한 피부 발진 임상 바이오마커 발굴의 초석이 될 것으로 생각된다.

핵심 되는 말: 간질, 라모트리진, 피부 발진, 약물유전체학, 전장 유전체 연관 분석

PUBLICATION LIST

1. Lee MK, **Kim SW**, Lee JH, Cho YJ, Kim DE, Lee BI, Kim HM, Lee MG, Heo K. A newly discovered LGI1 mutation in Korean family with autosomal dominant lateral temporal lobe epilepsy. Seizure 2014;23(1):69-73

2. **Kim SW**, Lee JH, Lee SH, Hong HJ, Lee MG, Yook KH. ABCB1 c.2677G>T variation is associated with adverse reactions of OROS-methylphenidate in children and adolescents with ADHD. J Clin Psychopharmacol 2013;33(4)491-8.

3. Koh KB, Choi EH, Lee YJ, Han MY, Choi SS, **Kim SW**, Lee MG. The relation of serotonin-related gene and COMT gene polymorphisms with criminal behavior in schizophrenic disorder. J Clin Psychiatry 201;73(2):159-63.

4. Lee JH, Kim KW, Gee HY, Lee J, Lee KH, Park HS, Kim SH, **Kim SW**, Kim MN, Kim KE, Kim KH, Lee MG, Sohn MH. A synonymous variation in protease-activated receptor-2 is associated with atopy in Korean children. J Allergy Clin Immunol 2011;128(6):1326-34.

5. Lee SH, **Kim SW**, Lee MG, Yook KH, Greenhill LL, Frandin KN, Hong HJ. Lack of association between response of OROS-methylphenidate and norepinephrine transporter (SLC6A2) polymorphism in Korean ADHD. Psychiatry Res. 2011;186(2-3):338-44.

6. Song JE, Yook KH, Lee SH, **Kim SW**, Lee MG, Hong HJ. Case-Control Association Study of the Norepinephrine Transporter Gene Polymorphism in Children with ADHD. 소아청소년정신의학회지 2010;21(1):23-30

7. Kim WJ, Lee JH, Yi J, Cho YJ, Heo K, Lee SH, **Kim SW**, Kim MK, Kim KH, In Lee B, Lee MG. A nonsynonymous variation in MRP2/ABCC2 is associated with neurological adverse drug reactions of carbamazepine in patients with epilepsy. Pharmacogenet Genomics 2010;20(4):249-56.

8. Lee SH, Lee MS, Lee JH, **Kim SW**, Kang RH, Choi MJ, Park SJ, Kim SJ, Lee JM, Cole SP, Lee MG. MRP1 polymorphisms associated with citalopram response in patients with major depression. J Clin Psychopharmacol 2010;30(2):116-25.

9. Kwak JO, Lee SH, Lee GS, Kim MS, Ahn YG, Lee JH, **Kim SW**, Kim KH, Lee MG. Selective inhibition of MDR1 (ABCB1) by HM30181 increases oral bioavailability and therapeutic efficacy of paclitaxel. Eur J Pharmacol 2010 10;627(1-3):92-8.

10. Gee HY, Kim CK, **Kim SW**, Lee JH, Kim JH, Kim KH, Lee MG. The L441P Mutation of CFTR and its Molecular Pathogenic Mechanisms in a Korean Patient with Cystic Fibrosis. J Korean Med Sci 2010;25(1):166-71.

11. Sohn MH, Lee JH, Kim KW, **Kim SW**, Lee SH, Kim KE, Kim KH, Lee

CG, Elias JA, Lee MG. Genetic variation in the promoter region of chitinase 3-like 1 is associated with atopy. Am J Respir Crit Care Med 2009;179(6):449-56.