Supplementary Appendix

Interaction Analysis

Accounting for non-independence between SNPs due to intermarker LD, SNPSpD (41) approximates the 66 replication SNPs to be equivalent to 48 independent SNPs, thus indicating a more reasonable correction would be for 1,128 interaction tests. The resulting less-conservative global p-value for the *KCNB2* SNP rs1431656 and *CACNB2* SNP rs7076100 interaction is P = 0.022. The second most significant interaction was also observed between a *KCNB2* SNP (rs1542709) and the same *CACNB2* SNP rs7076100 (pointwise P = 0.0000533; less-conservative global P = 0.060). However, *KCNB2* SNPs rs1431656 and rs1542709 are in strong LD (r^2 = 0.79), indicating the weaker rs1542709 × rs7076100 interaction is simply due to LD between rs1431656 and rs1542709. All other interaction tests were not significant producing pointwise p-values >0.0004 (less-conservative global P > 0.45).

Compared to the respective individual odds ratios of 0.77 and 0.86 conferred by the minor alleles of rs1431656 (G) and rs7076100 (A) (Table 3), the odds ratio for the rs1431656 × rs7076100 interaction is 0.61. In terms of their major alleles, rs1431656 (A), rs7076100 (T) and the rs1431656 × rs7076100 interaction confer an increase of migraine in the Finnish case-control sample with odds ratios of 1.30, 1.16 and 1.64, respectively. Importantly, rs1431656 and rs7076100 alleles are completely uncorrelated (r^2 = 0.0009) in the Finnish sample, indicating their interaction associated with migraine incidence is not resulting from spurious or coincidental correlation.

Both rs1431656 and rs7076100 were successfully genotyped in the Brisbane and Leiden replication samples. Although the case allele frequencies for both rs1431656 and rs7076100 were very similar across the three tested samples, only rs7076100 in the Brisbane sample produced a marginally significant association result (pointwise P = 0.057) (see Table 6 for comparison of population allele frequencies). Not surprisingly, due to the lack of evidence for association between migraine and both rs1431656 or rs7076100 in the Brisbane and Leiden samples, the rs1431656 ×

rs7076100 interaction observed in the Finnish sample, or any other interaction between any other of the intragenic variants in these two genes was observed.

To further investigate SNP × SNP epistasis between *KCNB2* and *CACNB2* SNPs, all inter-genic interactions were tested in the replication cohorts. SNPSpD estimated the 12 genotyped *KCNB2* SNPs were equivalent to 6 completely independent SNPs, while the 7 *CACNB2* SNPs genotyped in the Brisbane and Leiden cohorts were equivalent to 6 independent SNPs, and the 4 *CACNB2* SNPs genotyped in the Cologne and Munich cohorts were equivalent to 3 independent SNPs. Therefore, allowing to intra-genic correlation between SNPs we estimate approximately 36 (6 × 6) and 18 (6 × 3) independent inter-genic interactions were tested in the Brisbane/Leiden and Cologne/Munich cohorts, respectively.

In the Leiden cohort, no inter-genic SNP × SNP epistasis was observed (i.e., all pointwise $P > 0.05$), while in the combined Brisbane cohort, only weak evidence for epistasis was observed between *CACNB2* SNP rs11014504 and *KCNB2* SNPs rs11782118 and rs13276133, producing pointwise p-values $P = 0.02228$ and $P = 0.03654$, respectively. Contrastingly, in the combined Cologne and Munich cohort, encouraging evidence for epistasis was observed between *CACNB2* SNP rs8181477 and *KCNB2* SNPs rs7006287 and rs11782118, producing pointwise $P = 0.004017$ and $P = 0.004454$, respectively. Although, given *KCNB2* SNPs rs7006287 and rs11782118 are in strong LD ($r^2 = 0.83$), the weaker rs8181477 × rs11782118 interaction is simply due to LD between rs7006287 and rs11782118. After correcting for multiple interaction tests, the rs8181477 × rs7006287 interaction just falls short of significance with a corrected global p-value of 0.072.

1. Nyholt, D.R. (2004) A Simple Correction for Multiple Testing for SNPs in Linkage Disequilibrium with Each Other. *Am J Hum Genet,* **74,** 765-769.