Ultra-rare genetic variation in the common epilepsies: a case-control sequencing study

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ABSTRACT

BACKGROUND

Despite progress in understanding the genetics of rare epilepsies, the more common epilepsies have proven less tractable to traditional gene-discovery analyses. We aimed to assess the contribution of ultra-rare genetic variation to the common epilepsies.

METHODS

We did a case-control sequencing study using the exome sequence data from unrelated individuals clinically evaluated for one of the two most common epilepsy syndromes: familial genetic generalized epilepsy (GGE) or familial sporadic non-acquired focal epilepsy (NAFE). Individuals were recruited between Nov 26, 2007 and Aug 2, 2013 through the multicentre Epilepsy Phenome/Genome Project and Epi4K collaborations, and were sequenced at the Institute for Genomic Medicine, Columbia University (New York City, USA) between Feb 6, 2013 and Aug 18, 2015. To identify epilepsy risk signals, we tested all protein-coding genes for an excess of ultrarare genetic variation among the cases compared to unrelated individuals of European ancestry selected for control purposes through unrelated studies.

FINDINGS

We separately compared the sequence data from 640 individuals with familial GGE and 525 individuals with familial NAFE to the same group of 3,877 controls, and found significant excess of ultra-rare deleterious variation in genes established as causative for dominant epilepsy disorders (familial GGE: OR 2.3 [95% CI 1.7–3.2]; p=9.1x10^{-8}) (familial NAFE: OR 3.6 [95% CI 2.7–4.9]; p=1.1x10^{-17}). Comparing an additional collection of 662 individuals with sporadic NAFE to controls did not identify study-wide significant signals. For the familial NAFE cases, we found that five previously known epilepsy genes ranked as the top five genes enriched for ultra-rare deleterious variation. After accounting for the control carrier rate we estimate that these five genes contribute to the risk of epilepsy in approximately 8% of familial NAFE cases. Our analyses showed that no individual gene was significantly associated with epilepsy; however, known epilepsy genes achieved lower p-values relative to the rest of the protein-coding genes (p=5.8x10^{-8}).

INTERPRETATION

We identified excess ultra-rare variation in known epilepsy genes, which establishes a clear connection between the genetics of common and rare severe epilepsies, and shows that the variants responsible for the observed epilepsy risk signal are exceptionally rare in the general population. Our results suggest that the emerging paradigm of targeting treatments to the genetic cause in rare devastating epilepsies may also extend to a proportion of common epilepsies. These findings might allow clinicians to broadly explain the aetiology of these syndromes to patients, and lay the foundation for possible precision treatments in the future.

FUNDING

National Institute of Neurological Disorders and Stroke (NINDS), Epilepsy-Research UK.

INTRODUCTION

Next generation sequencing has proven successful in identifying genetic contributions to rare Mendelian disorders and cancers,[1, 2] creating widespread optimism that treatments can be targeted to underlying causes of disease.[3] Although epilepsy is a common complex disease, it is emerging as a group of disorders with precision medicine opportunities similar to those in rare Mendelian disorders and cancers.[4] Unlike many common diseases, epilepsy genetics research is identifying not only the genes responsible, but also the genetic variants contributing to disease in individual patients. This is most apparent in the role of *de novo* mutations in the epileptic encephalopathies.[5, 6]

Traditional heritability studies of the common epilepsies consistently show strong genetic effects in non-acquired focal epilepsy (NAFE) and in genetic generalized epilepsy (GGE), with both shared and distinct genetic contributions to these broadly defined epilepsies.[7, 8] Two important unresolved questions are the extent to which the genes responsible for rare severe epilepsies contribute to common epilepsies, and whether, as in the rare epilepsies, genetic risk arises primarily from ultra-rare variants of large effect including *de novo* mutations,[5, 6] or from a constellation of common variants each conferring small or modest effect.[9-13]

Exome sequencing of large case and control cohorts followed by genome-wide collapsing analyses provide a hypothesis-free approach to discovering novel disease genes and better understanding the overall contribution of ultra-rare genetic variation to disease.[14] Here, we assess the contribution of ultra-rare genetic variation to common epilepsies while controlling for background variation in the general population.

METHODS

Participants

For this case-control study, participants with familial or sporadic NAFE or familial GGE were recruited between November 26, 2007 and August 2, 2013 through the international Epilepsy Phenome/Genome Project (EPGP) and Epi4K collaborations (appendix), as previously described.[15] The case samples were sequenced between February 6, 2013 and August 18, 2015 by the Institute for Genomic Medicine, Columbia University (New York City, NY, USA). To be clinically classified as having NAFE, patients were required to have focal seizures and no evidence of an epileptogenic lesion on clinical imaging; however, hippocampal sclerosis was not considered an exclusion criterion. To be clinically classified as having GGE, patients were required to have a diagnosis of generalized epilepsy with absence, myoclonic or tonic-clonic seizures and generalized spike-and-wave on an EEG, and no or mild intellectual disability. All patients were clinically evaluated by their local clinician or the clinical team at recruiting centres. Individuals with unclassifiable epilepsy or classified as having both GGE and NAFE were excluded from the analyses.

To be classified as a familial case, at least one reported relative (up to third degree) who had been diagnosed with epilepsy was required. The sporadic NAFE cohort included participants who self-reported no known epilepsy family history and were recruited from international hospital, outpatient, and epilepsy clinics (appendix).[15, 16] Written informed consent was collected at the time of recruitment at each of the clinical sites. Patient collection and sharing of anonymised specimens for research was approved by site-specific Institutional Review Boards and ethic committees.

The control cohort comprised of unrelated individuals of European ancestry that had been selected for control purposes and sequenced through unrelated studies not focused on neurodevelopmental, neuropsychiatric or severe paediatric disease (appendix).

Procedures

Sequencing was performed at the Institute for Genomic Medicine, Columbia University (New York City, NY, USA). Samples were exome sequenced using the Agilent All Exon (50MB or 65MB; Agilent Technologies, Santa Clara, CA, USA) or the Nimblegen SeqCap EZ V2.0 or 3.0 Exome Enrichment kit (Roche NimbleGen, Madison, WI, USA) or whole genome sequenced using HiSeq 2000 or 2500 (Illumina, San Diego, CA, USA) sequencers according to standard protocols.

The sequence data from patients with epilepsy and controls were processed using the same Institute for Genomic Medicine bioinformatics pipeline (appendix). We focused on 18,668 consensus coding sequence (CCDS; release 14) protein-coding genes. On average, at least 10-fold coverage was achieved for 95.8% (familial GGE), 96.8% (familial NAFE), 97.1% (sporadic NAFE) and 95.6% (controls) of the 33.27 Mbps of the CCDS. For each protein-coding site in the CCDS—inclusive of two base intronic extensions to accommodate canonical splice variants—we determined the percentage of cases and controls that had ≥10-fold coverage at the site. To alleviate confounding due to differential coverage we used a site-based pruning strategy similar to our previously described exon-pruning strategy.[17] Individual CCDS sites were excluded from analysis if the absolute difference in the percentage of the cases compared to controls with adequate coverage of the site differed by greater than 5.19% (familial GGE vs. controls), 5.14% (familial NAFE vs. controls) and 6.39% (sporadic NAFE vs. controls) (appendix). Site-based pruning resulted in 8.9% (GGE), 8.3% (familial NAFE) and 8.3% (sporadic NAFE) of the CCDS bases excluded from the respective analyses to alleviate issues from differential coverage. Thus, all gene tests were performed on the pruned CCDS where cases and controls had similar opportunity to call gene variants (appendix).

STATISTICAL ANALYSIS

To search for genes that confer risk for common epilepsy syndromes, we implemented a genic collapsing analysis,[17] in which only a single affected individual (the index case) from each family was included. We applied standard procedures to address potential bias due to relatedness and population stratification (appendix). The analyses focused on CCDS protein-coding sites with minimal variability in coverage between the case and control populations.

As in our earlier work,[17] the term "qualifying variants" has been adopted to refer to the subset of variation within the sequence data that meets specific criteria designed to enrich for pathogenic variants. We defined qualifying variants in four ways (Table 1). Our primary analysis focused on ultra-rare variants where a combination of internal (the test samples) and external data (the Exome Variant Server [EVS][18] and Exome Aggregate Consortium [ExAC; release 0.3][19]). The test cohort was used to identify variants with a minor allele frequency (MAF) <0.05% among our combined case and control population being tested. The EVS and ExAC external databases were used to identify variants found among the test samples and absent (i.e., MAF=0%) among the two external reference control cohorts. The MAF was set to <0.05% in the combined case and control test collection to accommodate the possibility of multiple instances of a risk variant among cases. The two freely available EVS and ExAC external databases were solely used to support the rarity of the identified variants and did not contribute as control samples to the tests themselves.

For the primary analysis, functional annotation focused on single nucleotide substitution and insertion or deletion variants annotated as having a loss-of-function, inframe insertion or deletion, or a "probably damaging" missense effect by PolyPhen-2 (HumDiv).[20] Three secondary analyses were performed to evaluate the contribution to epilepsy risk from: rare loss-of-function variants with an internal and external population MAF up to 0.1%; rare non-synonymous variation in the general population with an internal and external MAF up to

143 0.1%; and a presumed neutral model that imposed similar MAF thresholds as our primary
144 analysis, but focused specifically on protein-coding variants predicted to have a synonymous
145 effect. The purpose of the presumed neutral model was to further confirm that no cryptic
146 factors might be increasing qualifying variant calling among one of the groups.

147 For each of the four models, we tested the complete list of 18,668 CCDS genes. For each
148 gene, an indicator variable (1/0 states) was assigned to each individual based on the presence
149 of at least one qualifying variant in the gene (state 1) or no qualifying variants in that gene
150 (state 0). We used a two-tailed Fisher's exact test to identify genes where there was a
151 significant enrichment of qualifying variants in the case or control group. To control for the
152 type-I error rate within each epilepsy phenotype, we defined study-wide significance as
153 $p=8.9x10^{-7}$, correcting for 18,668 CCDS genes studied across three models (0.05/[3x18668]).
154 We did not correct for the neutral control model.

155 All collapsing analyses were performed using an in-house package, Analysis Tool for
156 Annotated Variants (ATAV). Binomial tests were used to evaluate whether there was an
157 enrichment of previously reported pathogenic variants among the case collection of
158 qualifying variants. Hypergeometric tests were performed to assess whether among the
159 collapsing analysis results the known epilepsy genes preferentially achieved lower p-values
160 relative to the rest of the genome. Cochran-Mantel-Haenszel tests were adopted to combine
161 the results of the gender stratified sex chromosome collapsing analyses.

162 We also used the primary analysis results from each of the patient groups to assess
163 enrichment among six biologically informed gene-sets that were chosen and described in our
164 earlier studies of the epileptic encephalopathies,[5, 21] including a list of 43 established
165 dominant epilepsy genes (appendix).[3] To account for background variation in gene-set tests
166 we applied a logistic regression model (appendix).

To assess the contribution to epilepsy risk coming from variants with increasing minor allele frequencies (MAF), we developed a multivariable logistic regression model that focuses on the known epilepsy genes and relates disease risk to the presence of variants among increasing MAF bins (appendix).

These additional binomial, hypergeometric, Cochran-Mantel-Haenszel, and logistic regression tests were completed using R package 'stats' version 3.2.2.

ROLE OF THE FUNDING SOURCE

The funders of the study had no role in study design, data collection, data analysis, data interpretation or writing of the report. The corresponding author had full access to the data in the study and had final responsibility for the decision to submit for publication.

RESULTS

We sequenced the exomes of 1,827 patients with epilepsy—640 unrelated individuals with a diagnosis of familial GGE and 525 unrelated individuals with a diagnosis of familial NAFE of European ancestry. We also sequenced an additional 662 individuals with sporadic NAFE. We compared these three groups of patients with epilepsy to 3,877 controls, who were unrelated individuals of European ancestry with no known epilepsy diagnosis.

Among our familial GGE cohort, no individual gene achieved study-wide significant enrichment for qualifying variants (Figure 1, appendix). Of the total 76,313 qualifying variants in the GGE primary analysis, 15.0% were found among cases in the familial GGE cohort. We then found that among the 76,313 qualifying variants, four unique variants overlapped a codon previously reported to have a pathogenic-classified epilepsy variant

based on the disease-associated variant catalogues of ClinVar, the Online Mendelian Inheritance in Man (OMIM), or the Human Gene Mutation Database (HGMD). All four variants (two *SCN1A,* one *GABRG2,* and one *SCN1B*; appendix) were found among the familial GGE cohort, an improbable enrichment given the expected proportion of 15.0% ($p=5.1x10^{-4}$, two-tailed exact binomial test). Through an evaluation of the scientific literature, these four cases were confirmed as unrelated to those families reported in the literature.

While no single gene attained study-wide significance in the familial GGE analysis, three known epilepsy genes (*KCNQ2*, *GABRG2*, and *SCN1A),* were among the top ten case-enriched genes in the primary analysis (Figure 1). A hypergeometric test was run at each of the gene ranks occupied by one of the 43 established epilepsy genes (appendix), and we found that the enrichment was greatest at rank 151 whereby seven of the 43 known epilepsy genes had been accounted for (hypergeometric $p=5.8x10^{-8}$; appendix).

When we assessed enrichment among six biologically informed gene-sets, we found that the familial GGE cohort had a significant enrichment of ultra-rare functional variation among 43 known dominant epilepsy genes ($p=9.1x10^{-8}$, OR=2.3 [95% C.I. 1.7–3.2]; Table 2) and a subset of 33 genes known to contribute to epileptic encephalopathy ($p=2.6x10^{-7}$, OR=2.6 [95% C.I. 1.8–3.6]).[3] We confirmed that the signal of enrichment for qualifying variants among known epilepsy genes was consistently greater than the control rate across groupings of the familial GGE cohort, reflecting the number of affected relatives (appendix). While they did not achieve study-wide significance (defined as $p< 8.9x10^{-7}$), we also investigated qualifying variant enrichment among the fragile X mental retardation protein associated genes,[22] the genes encoding the NMDA receptor (NMDAR), and neuronal activity-regulated

cytoskeleton-associated protein, postsynaptic signalling complexes,[23] mouse seizure-associated orthologs,[24] and ion channel protein-coding genes[25] (Table 2). None of these gene-set tests reported enrichment of neutral variation.

Among the primary analysis of our familial NAFE cohort (figure 2A), *DEPDC5* achieved study-wide significance (OR 8.1 [95% C.I. 3.6–18.3], $p=1.8x10^{-7}$). *LGI1* did not achieve study-wide significance (OR 29.9 [95% C.I. 6.0–288.0], $p=1.4x10^{-6}$). Established epilepsy genes *PCDH19* (OR 22.4 [95% C.I. 4.0–226.4], $p=6.4x10^{-5}$), *SCN1A* (OR 5.5 [95% C.I. 2.3–12.9], $p=9.0x10^{-5}$) and *GRIN2A* (OR 7.5 [95% C.I. 2.2–25.1], $p=5.3x10^{-4}$) occupied the 3rd – 5th genome-wide ranks (appendix), but were not study-wide significant after correcting for the 56,004 tests (Bonferroni corrected p = 1). A hypergeometric test indicated that it was highly improbable for five of the 43 known dominant epilepsy genes to occupy the top five positions of the primary analysis by chance (*p*=5.7x10-14) (appendix).

Of 74,272 qualifying variants identified in the primary analysis of 525 individuals with familial NAFE and 3,877 controls, 9,092 (12.2%) of these were found among the familial NAFE cases. Among the 74,272 qualifying variants, nine variants overlapped a codon of a ClinVar, OMIM, or HGMD literature-reported pathogenic variant in a confirmed unrelated family. All nine unique variants (three *DEPDC5,* three *PCDH19,* one *CHRNB2,* one *GRIN2A* and one *LGI1* variant; appendix) were found among nine distinct NAFE cases of the combined 4,402 unrelated samples used in the familial NAFE collapsing analysis, despite the expected proportion being 12.2% (exact binomial test $p=6.2x10^{-9}$).

The known dominant epilepsy gene-set (OR=3.6 [95% CI 2.7–4.9], $p=1.1x10^{-17}$) and the epileptic encephalopathy gene-set (OR=3.3 [95% CI 2.3–4.7], $p=5.0x10^{-11}$) were study-wide significantly enriched for qualifying variants among the primary analysis of familial NAFE cases (Table 2). As observed in the familial GGE cases, the signal of enrichment for

qualifying variants among known epilepsy genes remained consistently greater than the control rate across groupings of the familial NAFE cohort stratified by the number of affected relatives (appendix). Presumably neutral variation was not significantly enriched among any gene-set. Under the loss-of-function model, *DEPDC5* achieved study-wide significance (OR=53.07, [95% C.I. 12.1–481.3], $p=9.6x10^{-12}$), with 14 (2.7%) of familial NAFE cases having a *DEPDC5* loss-of-function variant compared to only two (0.05%) controls. Focusing solely on PolyPhen-2 'probably damaging' missense *DEPDC5* qualifying variants showed that they were non-significant for enrichment (3 [0.6%] of 525 cases vs. 12 [0.3%] of 3877controls; OR=1.9 [95% C.I. 0.3–6.9], p=0.41; Figure 2B and appendix). Results from the list of 43 known dominant epilepsy genes that achieved an uncorrected $p<0.05$ in the primary or loss-of-function models are listed in the appendix.

Sanger sequencing was used to validate a subset of qualifying variants found among 19 established and 13 candidate epilepsy genes (appendix). Our rate of Sanger validation was 97.0% (128/132) of the qualifying variants identified through the collapsing tests (appendix). When available, we also Sanger sequenced qualifying variants among affected first-degree relatives of index cases used in the collapsing analyses. We looked at six genes where we had enough affected first-degree relatives to be sufficiently powered to achieve an uncorrected $p<0.05$ from a test of preferential segregation (appendix). Comparing to the expected rate of 50%, *SCN1A* (88.2% co-occurrence; $p=1.2x10^{-3}$), *DEPDC5* (100% co-occurrence; $p=4.9x10^{-4}$) and *GRIN2A* (100% co-occurrence; $p=7.8x10^{-3}$) had significant co-occurrence among affected first-degree family members, after correcting for the six studied genes (adjusted $\alpha=8.3x10^{-3}$; appendix).

To explore which variants, as a function of MAF, are most important to the observed risk signal we performed conditional analyses (appendix). These analyses show that among the observed epilepsy risk signal, beyond the ultra-rare qualifying variants (i.e., absent in EVS and ExAC) there is no significant contribution from variants with minor-allele frequencies up to 0.1% population MAF. This was true for both the familial GGE and familial NAFE populations (Figure 3; appendix).

Comparing 662 sporadic NAFE cases to controls did not identify study-wide significant genes across any of the three models (appendix). Of the five previously described familial NAFE top ranked genes, we found that only *LGI1* achieved an uncorrected p-value of less than 0.05, (OR 8.8 [95% C.I. 1.0–105.7], p=0.025). None of the tested gene-sets were significantly enriched with qualifying variants among sporadic NAFE cases (Table 2, Figure 3).

DISCUSSION

In this study, we demonstrate the presence of clear genetic risk signal for common epilepsies across genes established as responsible for familial and rare severe epilepsies. In our analysis of a cohort of individuals with familial NAFE, we found that five established epilepsy genes (*DEPDC5, LGI1, PCDH19, SCN1,A* and *GRIN2A*) occupy the top five positions genome-wide, and after correcting for background variation, the collection of these five genes contribute to approximately 8% of patients with familial NAFE. Sampling from a similarly sized familial GGE collection identified three established epilepsy genes (*KCNQ2, SCN1A,* and *GABRG2*) ranking among the top ten genes. Power estimates highlight the potential for new epilepsy gene discovery using this framework on larger sample sizes (appendix). Using

288 the example from *LGI1*, while we found only two qualifying variants among 3,877 controls
289 (0.05%), identifying eight familial NAFE case carriers in the primary analysis (1.5% of the
290 familial NAFE cohort) was still inadequate to achieve study-wide significance ($p<8.9x10^{-7}$)
291 for this known familial NAFE gene. Assuming the sampled rates for *LGI1* case and control
292 carriers remain the same, we estimate that *LGI1* would achieve study-wide significance with
293 the inclusion of approximately twice as many controls and 70 more unrelated familial NAFE
294 cases.

295 As in earlier studies, our data show that *SCN1A* contributes to risk in both the familial GGE
296 and familial NAFE epilepsy cohorts[11] and this enrichment is not explained by diagnoses of
297 generalized epilepsy with febrile seizures plus (GEFS+). *SLC9A2* was also among the top 20
298 genes in both the familial NAFE and familial GGE cohort analyses; however, it did not reach
299 study-wide significance.No clear risk signal for epilepsy was found among the sporadic
300 NAFE cohort. This might be explained by the possibility that non-genetic (acquired) causes
301 play a more important role among individuals with sporadic NAFE, leading to substantially
302 reduced power but otherwise similar genetics. Other unexplored genetic contributions to the
303 sporadic NAFE cohort include somatic mutations arising later in development, limited to the
304 brain or at undetectable levels in blood-extracted DNA using conventional whole-exome
305 sequencing.

306 Among the most important findings in this work is our ability to identify clear risk signal in
307 these data and subsequently show that the observed risk signal is concentrated among the
308 rarest variants in the human population. In fact, among the 43 established dominant epilepsy
309 genes we have shown that there is no evidence of risk contribution from variants observed at
310 greater than 0.005% allelic frequency. This, however, does not preclude any other
311 contributions to risk being present among currently unrecognized epilepsy risk genes. This
312 work not only illustrates the value of large reference control variant databases,[19] but provides

clinically relevant information concerning the frequency spectrum of risk variants for a common complex disease.

A new paradigm is emerging for the treatment of rare devastating epilepsies, where treatments are being targeted to the precise genetic cause of disease.[3, 26-28] For example, children with *KCNT1* gain-of-function mutations have been treated with quinidine[27, 29] while patients with *GRIN2A* gain-of-function mutations have been treated with memantine, a specific NMDA receptor blocker.[28, 30] As this paradigm becomes more established, a critical question for the field is whether the approach will also apply to common epilepsies. If so, the field, which is currently accustomed to undertaking large randomised controlled trials in broad phenotypes, needs to rapidly develop a framework for classification based on ultra-rare variants in what is effectively a collection of rare genetic diseases. The work presented here demonstrates that many genes responsible for devastating rare and familial epilepsies also contribute to more common epilepsies, and it is still the ultra-rare variants that are relevant in those genes. This suggests that the emerging precision medicine paradigm of targeting treatments to the underlying causes of disease in the rarest epilepsies may also find application among the common epilepsies.

RESEARCH IN CONTEXT

Evidence before this study

The genetic underpinnings of the common epilepsies are largely unknown, especially the relative contributions of common variants of small effect size versus rare variants of large effect, where opportunities for novel therapeutic strategies may be greater. We searched PubMed for the terms "exome sequencing" and "common epilepsy" for reports published before June 28, 2016, with no language restrictions. There were no reports of exome sequencing of large case collections of common complex epilepsies. Although exome sequencing studies have been successful in implicating numerous genes and finding the relevant mutations for individuals with rare severe paediatric epilepsies, including epileptic encephalopathies, estimating the risk contribution from the ultra-rare protein-coding variants has been less clear for many of the common epilepsy syndromes.

Added value of this study

We used whole-exome sequencing on a large collection of two common epilepsy syndromes, genetic generalized epilepsy (GGE) and non-acquired focal epilepsy (NAFE), to search for an excess of ultra-rare deleterious qualifying variants, and compared the qualifying variant rates found among cases to background rates estimated from sequenced controls. Among familial index cases sampled from the common epilepsies, we found a significant excess of ultra-rare deleterious variation within known epileptic encephalopathy genes. We also demonstrate that the epilepsy risk signal observed in the known epilepsy genes is accounted for by the ultra-rare class of variants that are absent among large reference control cohorts, such as ExAC and EVS. Variants in known epilepsy genes that were predicted to be deleterious, but found at very low frequencies among the population reference cohorts, showed no evidence of contribution to the observed epilepsy risk signal.

353 **Implications of all the available evidence**

354 The present findings provide three key conclusions important to our understanding of the
355 common epilepsies. First, identifying significant enrichment of ultra-rare deleterious variants
356 among established epilepsy genes illustrates that there are genuine signals to be found using
357 the analysis framework presented here. Secondly, we showed that the precision medicine
358 framework that is emerging for rare epilepsies can be expected to find applications among
359 more common epilepsies. Finally, we showed that the risk signals among the common
360 complex forms of epilepsy come from the rarest variants in the human population, providing
361 the clearest insight currently available into the genetic variants underlying this common
362 complex disorder. Further research is warranted to understand to what extent these findings
363 can be applied to clinical practice.

364

365

CONTRIBUTORS

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DECLARATION OF INTERESTS

DBG reports personal fees from Pairnomix and EpiPM /Clarus outside the submitted work. DA reports grants from NINDS furing the conduct of the study. EA reports grants from NINDS/NIH during the conduct of the study, and grants from Sunovion Pharmaceuticals Inc. outside the submitted work. FA reports grants from NINDS/NIH during the conduct of the study, and grants from Sunovion Pharmaceuticals Inc. outside the submitted work. SFB

reports grants from National Health and Medical Research Council and NINDS during the conduct of the study; grants from UCB Pharma, Novartis Pharmaceuticals, Sanofi-Aventis, Jansen Cilag, outside the submitted work; In addition, SFB has a patent for SCN1A testing held by Bionomics Inc and licensed to various diagnostic companies. No financial return although was a consultant to Bionomics and Athena diagnostics over 4 years ago; Patent pending for DEPDC5 testing applied for by University of South Australia and University of Melbourne. JLC reports personal fees from Shire, and grants from UCB Pharma, outside the submitted work. PC reports grants from NINDS (NS053998) during the conduct of the study. OD served on the scientific advisory board of Pairnomix during the conduct of the study. DD reports grants from NIH/NINDS during the conduct of the study. MPE reports grants from NIH/NINDS during the conduct of the study. EBG reports grants and personal fees from Neuropace Inc outside the submitted work. TG reports grants from NIH (NS053998) during the conduct of the study, and personal fees from AssureX Health, outside the submitted work; also has a patent (PCT/US2006/045631) with royalties paid by AssureX Health. EHK served on the Data Safety Board of GW Pharma, served on the Scientific Advisory Board of Atkins Nutritionals, and reports grants from Nutricia outside the reported work. RK reports grants from NINDS (NS053998) during the conduct of the study. HCM reports grants from NINDS/NIH during the conduct of the study. RO reports other from Trigeminal Solutions Inc outside the submitted work. KP reports grants from NIH during the conduct of the study. SPetrou reports equity from Pairnomix outside the submitted work; a patent for the diagnosis and treatment of epilepsy (WO2004/085674) issued to Bionomics Ltd, and a patent for treatment and diagnosis of epilepsy by detecting mutations in the SCN1A gene(WO2006/133508) issued to Bionomics Ltd. SPetrov reports interests in Pairnomix outside the submitted work. IES reports grants from NHMRC and NIH during the conduct of the study; personal fees from UCB, Transgenomics, GlaxoSmithKline, Sanofi, and Eisai outside the submitted work; patent for Epilepsy and Mental Retardation Limited to Females

with royalties paid (WO/2009/086591), and a patent for treatment, and diagnosis of epilepsy by detecting mutations in the SCN1A gene licensed (WO/2006/133508). RAS reports grants from NIH, Pediatric Epilepsy Research Foundation, and American Sleep Medicine Foundation outside the submitted work; reports personal fees from UpToDate outside the submitted work. EHS reports personal fees from Invitae outside the submitted work. JJS reports grants from NIH during the conduct of the study. SS reports personal fees from Accorda, AstraZenica, Neurelis, Questcor, Upsher Smith, and Xeris outside the submitted work. LLT reports grants from NIH, Epilepsy Study Consortium, and Eisai outside the submitted work. RT has received personal fees for speaking from UCB Pharma and Eisai outside the submitted work; personal fees for consulting from Sanofi outside the submitted work.

All other authors declare no competing interests.

ACKNOWLEDGEMENTS

We are grateful to the patients, their families, clinical research coordinators and referring physicians for participating in the various recruitment sites that provided the phenotype data and DNA samples used in this study. We thank the following professional and lay organizations for substantial assistance in publicizing EPGP and therefore enabling us to recruit participants effectively: AED Pregnancy Registry, American Epilepsy Society, Association of Child Neurology Nurses, California School Nurses Organization, Child Neurology Society, Citizens United for Research in Epilepsy, Epilepsy Alliance of Orange County, Epilepsy Foundation, Epilepsy Therapy Project, and Finding a Cure for Epilepsy and Seizures. We thank the EPGP Administrative Core (K.F. Schardein, R. Fahlstrom, S. Cristofaro and K. McGovern), EPGP Bioinformatics Core (G. Nesbitt, K. McKenna, V. Mays), staff at the Coriell Institute – NINDS Genetics Repository, and members of the Institute for Genomic Medicine (C. Malone, B. Krueger, S. Kamalakaran, C. Davidson, B.

441 Copeland, S. Kisselev, D. Collado, D. Fernandez, J. Charoensri and P. Cansler) for their
442 dedication and commitment to this work. We also thank R. Stewart, K. Gwinn, R. Corriveau,
443 B. Fureman and V. Whittemore from the National Institute of Neurological Disorders and
444 Stroke for their careful oversight and guidance of both EPGP and Epi4K. This work was
445 supported by grants from the National Institute of Neurological Disorders and Stroke (The
446 Epilepsy Phenome/Genome Project NS053998; Epi4K—Administrative Core NS077274;
447 Epi4K—Sequencing, Biostatistics and Bioinformatics Core NS077303; Epi4K—Multiplex
448 Families & Pairs Project NS077367 and Epi4K—Phenotyping and Clinical Informatics Core
449 NS077276) and project grant (P1104) to R.H.T. from Epilepsy-Research UK.

450

451 The authors would like to thank the NHLBI GO Exome Sequencing Project and its ongoing
452 studies which produced and provided exome variant calls for comparison: the Lung GO
453 Sequencing Project (HL-102923), the WHI Sequencing Project (HL-102924), the Broad GO
454 Sequencing Project (HL-102925), the Seattle GO Sequencing Project (HL-102926) and the
455 Heart GO Sequencing Project (HL-103010). The authors would like to thank the Exome
456 Aggregation Consortium and the groups that provided exome variant data for comparison. A
457 full list of contributing groups can be found at http://exac.broadinstitute.org/about.

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Cohort	Model	Internal MAF(%)	External MAF(%)	Variant Effects	# Genes with >0 qualifying variant(s)	CCDS represented in the tests (%)
Familial GGE	Primary^	0.05%	0%	LoF inframe insertions or deletions PolyPhen-2 (HumDiv) “probably” damaging	15,515	30.3Mbp (91.1%)
	LoF	0.1%	0.1%	LoF	10,712	
	Common (0.1% MAF)	0.1%	0.1%	LoF inframe insertions or deletions PolyPhen-2 (HumDiv) “probably” damaging	17,118	
	Presumed Neutral	0.05%	0%	Synonymous substitution	14,959	
Familial NAFE	Primary^	0.05%	0%	LoF inframe insertions or deletions PolyPhen-2 (HumDiv) “probably” damaging	15,438	30.5Mbp (91.7%)
	LoF	0.1%	0.1%	LoF	10,601	
	Common (0.1% MAF)	0.1%	0.1%	LoF inframe insertions or deletions PolyPhen-2 (HumDiv) “probably” damaging	17,089	
	Presumed	0.05%	0%	Synonymous substitution	14,871	

	Neutral					
Sporadic NAFE	Primary^	0.05%	0%	LoF inframe insertions or deletions PolyPhen-2 (HumDiv) “probably” damaging	15,507	30.5Mbp (91.7%)
	LoF	0.1%	0.1%	LoF	10,729	
	Common (0.1% MAF)	0.1%	0.1%	LoF inframe insertions or deletions PolyPhen-2 (HumDiv) “probably” damaging	17,108	
	Presumed Neutral	0.05%	0%	Synonymous substitution	14,956	

Table 1. Qualifying variant criteria in the four models.

^Primary analysis permits minor allele frequency (MAF) to be up to 0.05% (i.e., up to four alleles in the combined case and control test population) to accommodate for possible recurrent pathogenic variants that might be relevant to multiple cases. GGE = genetic generalized epilepsy. NAFE = non-acquired focal epilepsy. LoF = loss-of-function. MAF = minor allele frequency. CCDS = consensus coding sequence

Group	Gene set	Number of genes	Average qualifying variants[a]	Qualifying variants enrichment p-value (Odds Ratio [95% CI])	Neutral variation enrichment p-value	Enrichment after removing the 43 epilepsy genes p-value
Familial GGE	Known	*43*	0.052	**$p = 9.1\text{x}10^{-8}$ (OR=2.3 [95% CI 1.7 - 3.2])**	*p = 0.86*	*N/A*
	Known (EE)	*33*	0.037	**$p = 2.6\text{x}10^{-7}$ (OR=2.6 [95% CI 1.8 - 3.6])**	*p = 0.34*	*N/A*
	Ion Channel	*209*	0.264	$p = 0.028$ (OR=1.2 [95% CI 1.0 - 1.5])	*p = 0.73*	*p = 0.21*
	FMRP	*823*	1.481	$p = 0.034$ (OR=1.3 [95% CI 1.0 - 1.6])	*p = 0.94*	*p = 0.04*
	NMDAR & ARC	*78*	0.067	$p = 0.004$ (OR=1.6 [95% CI 1.1 - 2.1])	*p = 0.80*	*p = 0.007*
	MGI Seizure	*235*	0.269	$p = 0.003$ (OR=1.3 [95% CI 1.1 - 1.6])	*p = 0.97*	*p = 0.17*
Familial NAFE	Known	*43*	0.055	**$p = 1.1\text{x}10^{-17}$ (OR=3.6 [95% CI 2.7 - 4.9])**	*p = 0.87*	*N/A*
	Known (EE)	*33*	0.037	**$p = 5.0\text{x}10^{-11}$ (OR=3.3 [95% CI 2.3 - 4.7])**	*p = 0.65*	*N/A*
	Ion Channel	*209*	0.264	$p = 1.9\text{x}10^{-4}$ (OR=1.5 [95% CI 1.2 - 1.8])	*p = 0.47*	*p = 0.05*
	FMRP	*823*	1.466	$p = 0.77$ (OR=1.0 [95% CI 0.8 - 1.2])	*p = 0.77*	*p = 0.38*
	NMDAR & ARC	*78*	0.061	$p = 0.43$ (OR=0.8 [95% CI 0.5 - 1.3])	*p = 0.62*	*p = 0.40*
	MGI Seizure	*235*	0.261	$p = 0.05$ (OR=1.2 [95% CI 1.0 - 1.5])	*p = 0.81*	*p = 0.87*
Sporadic NAFE	Known	*43*	0.045	$p = 0.27$ (OR=1.2 [95% CI 0.8 - 1.8])	*p = 0.27*	*N/A*
	Known (EE)	*33*	0.030	$p = 0.79$ (OR=0.9 [95% CI 0.5 - 1.5])	*p = 0.49*	*N/A*
	Ion Channel	*209*	0.251	$p = 0.34$ (OR=0.9 [95% CI 0.7 - 1.1])	*p = 0.88*	*p = 0.25*
	FMRP	*823*	1.461	$p = 0.95$ (OR=1.0 [95% CI 0.8 - 1.2])	*p = 0.92*	*p = 0.94*
	NMDAR & ARC	*78*	0.063	$p = 0.65$ (OR=1.1 [95% CI 0.8 - 1.5])	*p = 0.49*	*p = 0.70*
	MGI Seizure	*235*	0.254	$p = 0.36$ (OR=0.9 [95% CI 0.7 - 1.1])	*p = 0.33*	*p = 0.33*

Table 2. Gene-set enrichment tests. P-values are from a logistic regression model that regresses the case/control status of a sample on the presence (1) or absence (0) of at least one qualifying variant among the corresponding gene set (Primary model). Reported p-values are uncorrected; the study-wide multiplicity-adjusted significance threshold $\alpha = 8.9\text{x}10^{-7}$. All tests use the individual's gender, exome-wide tally of qualifying variants, and the individual's gene-list-specific tally of rare neutral (synonymous) variation as correction factors (appendix). **Known** = 43 established dominant human epilepsy genes.[3] **Known (EE)** = A subset of genes securely implicated with epileptic encephalopathies. **Ion Channel** = genes coding for ion

channels.[25] **FMRP** = fragile X mental retardation protein associated genes.[22] **NMDAR & ARC** = NMDA receptor and neuronal activity-regulated cytoskeleton-associated protein synaptic transmission genes .[23] **MGI Seizure** = mouse orthologs linked with seizure phenotypes in the Mouse Genome Database.[24] [a]Average number of qualifying variants in the corresponding gene set, per sample in the test population.

Figure 1: Familial GGE primary model analysis. 15,515 genes had at least one case or control carrier (table 1). Qualifying variants were defined as a minor allele frequency <0.05% in internal case and control, and absent among external reference cohorts. Variants are annotated as loss-of-function, inframe insertions or deletions, or missense predicted to be "probably damaging" by PolyPhen-2 (HumDiv). No gene achieved study-wide significance (adjusted $\alpha < 0.05/[18668 * 3] = 8.9x10^{-7}$).

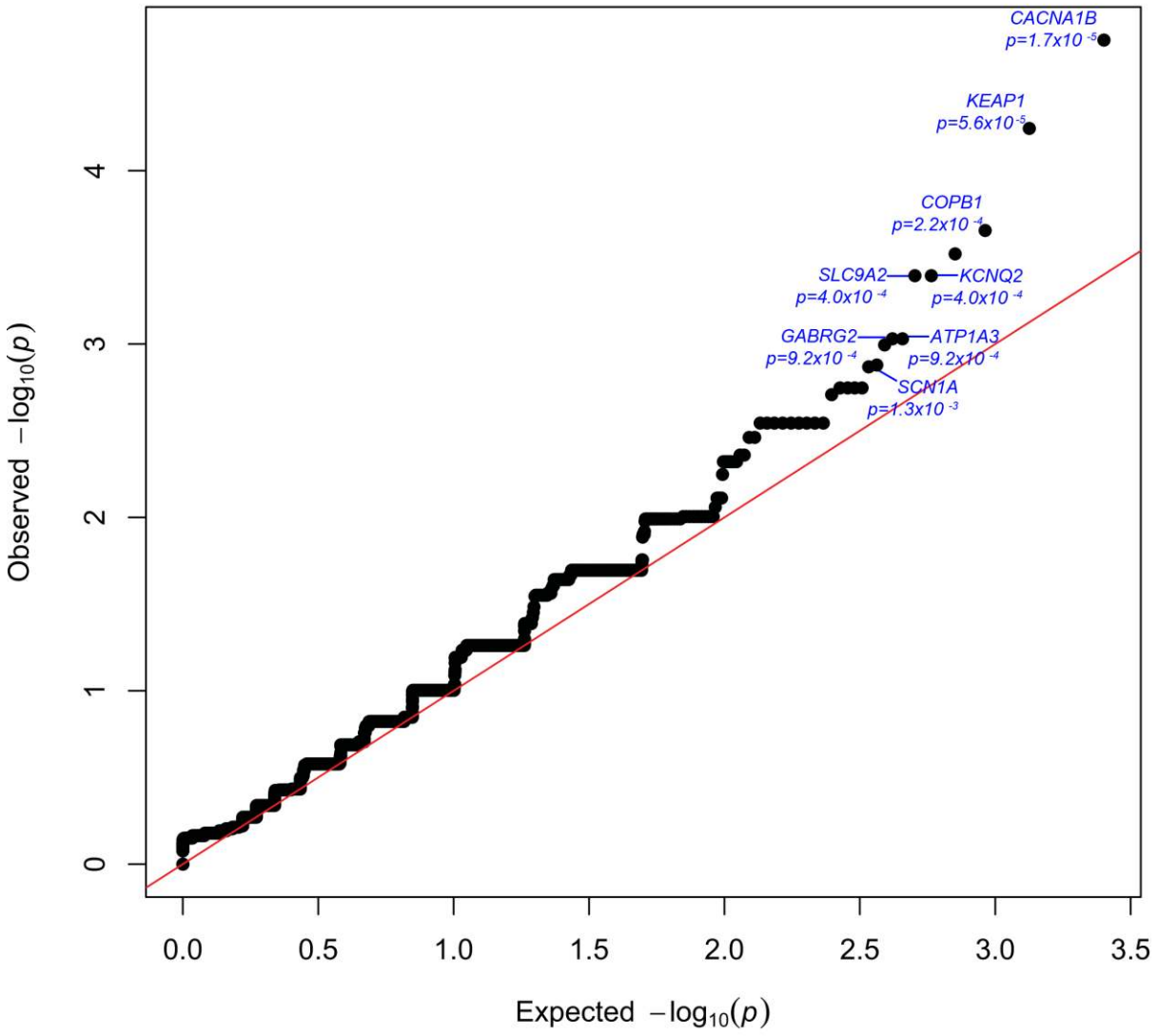


Figure 2: Familial NAFE primary model analysis. (A) 15,438 genes had at least one case or control carrier (table 1). Qualifying variants have a minor allele frequency <0.05% in internal case and control, and are absent among external reference cohorts. Variants are annotated as loss-of-function, inframe insertions or deletions, or missense predicted to be "probably damaging" by PolyPhen-2 (HumDiv). Only *DEPDC5*, achieved study-wide significance (adjusted $\alpha < 0.05/[18668 * 3] = 8.9x10^{-7}$). **(B)** 10,601 genes had at least one case or control carrier (table 1). Qualifying variants are variants with a population MAF<0.1% and annotated as loss-of-function effects. Only *DEPDC5* achieved study-wide significance.

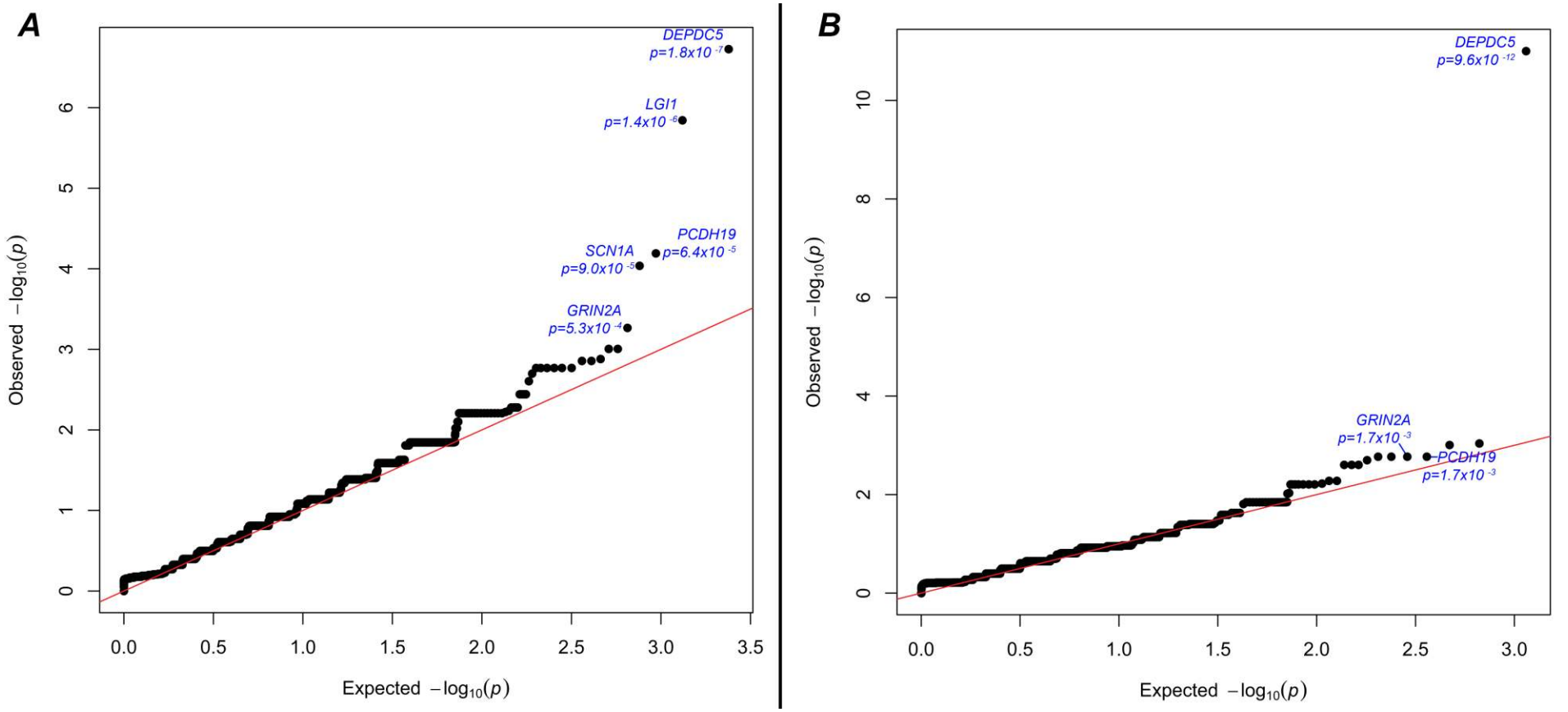
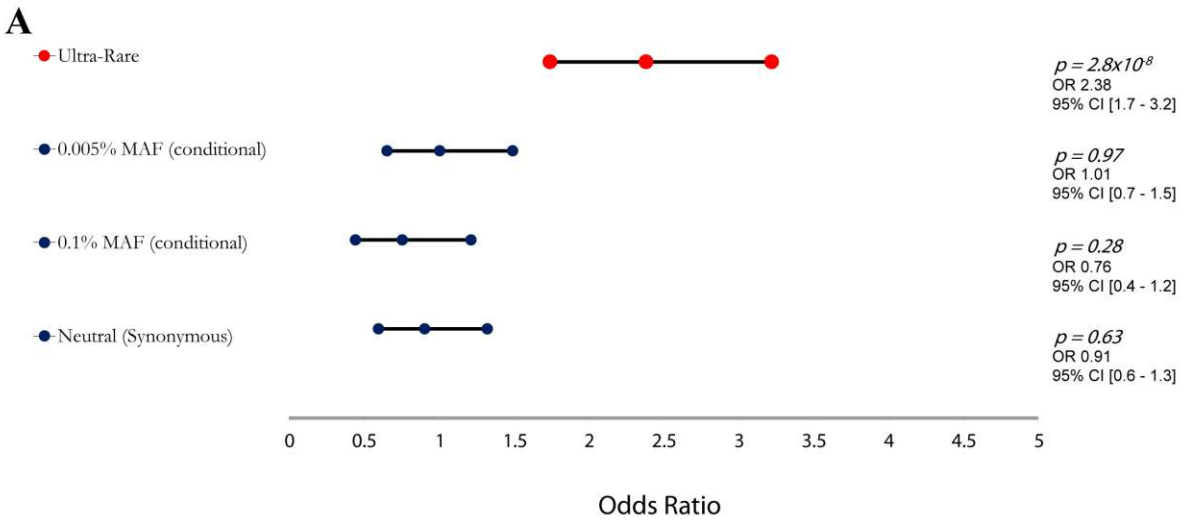
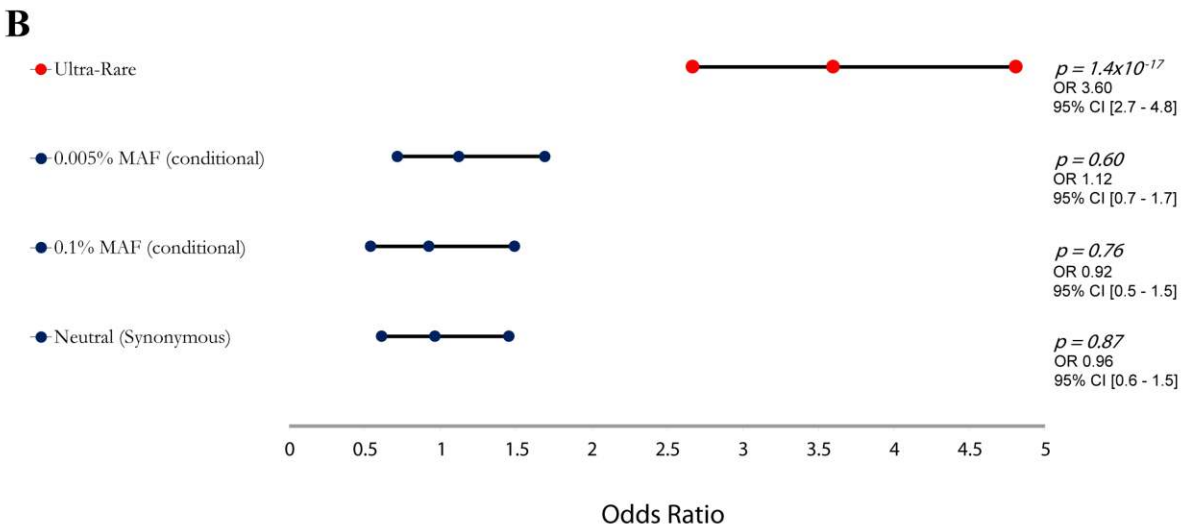
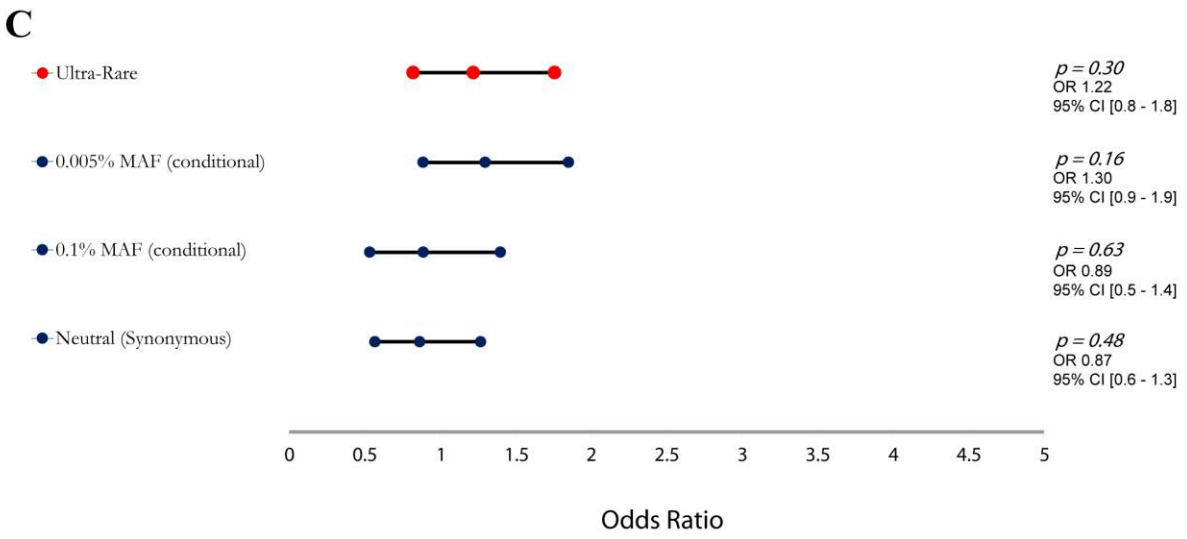


Figure 3: Enrichment of qualifying variants among 43 known epilepsy genes across increasing minor allele frequency bins. The ultra-rare variation bin reflects qualifying variants from the primary analyses. The 0.005% MAF (conditional) bin represents qualifying variants with a MAF greater than 0% but no greater than 0.005% in ExAC. The 0.1% MAF (conditional) bin represents qualifying variants with a MAF greater than 0.005% but no greater than 0.1% in ExAC. The neutral (synonymous)bin represents ultra-rare putatively neutral variants across the 43 epilepsy genes. Multivariate conditional analyses for the **(A)** familial GGE population **(B)** familial NAFE population **(C)** sporadic NAFE


A
Ultra-Rare
0.005% MAF (conditional)
0.1% MAF (conditional)
Neutral (Synonymous)
p = 2.8x10-8
OR 2.38
95% CI [1.7 - 3.2]
p = 0.97
OR 1.01
95% CI [0.7 - 1.5]
p = 0.28
OR 0.76
95% CI [0.4 - 1.2]
p = 0.63
OR 0.91
95% CI [0.6 - 1.3]
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5
Odds Ratio


B
Ultra-Rare
0.005% MAF (conditional)
0.1% MAF (conditional)
Neutral (Synonymous)
p = 1.4x10-17
OR 3.60
95% CI [2.7 - 4.8]
p = 0.60
OR 1.12
95% CI [0.7 - 1.7]
p = 0.76
OR 0.92
95% CI [0.5 - 1.5]
p = 0.87
OR 0.96
95% CI [0.6 - 1.5]
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5
Odds Ratio


C
Ultra-Rare
0.005% MAF (conditional)
0.1% MAF (conditional)
Neutral (Synonymous)
p = 0.30
OR 1.22
95% CI [0.8 - 1.8]
p = 0.16
OR 1.30
95% CI [0.9 - 1.9]
p = 0.63
OR 0.89
95% CI [0.5 - 1.4]
p = 0.48
OR 0.87
95% CI [0.6 - 1.3]
0 0.5 1 1.5 2 2.5 3 3.5 4 4.5 5
Odds Ratio

566

CONSORTIA

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