

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

25 We separately compared the sequence data from 640 individuals with familial GGE and 525
26 individuals with familial NAFE to the same group of 3,877 controls, and found significant
27 excess of ultra-rare deleterious variation in genes established as causative for dominant
28 epilepsy disorders (familial GGE: OR 2.3 [95% CI 1.7–3.2]; $p=9.1\times 10^{-8}$) (familial NAFE:
29 OR 3.6 [95% CI 2.7–4.9]; $p=1.1\times 10^{-17}$). Comparing an additional collection of 662
30 individuals with sporadic NAFE to controls did not identify study-wide significant signals.
31 For the familial NAFE cases, we found that five previously known epilepsy genes ranked as
32 the top five genes enriched for ultra-rare deleterious variation. After accounting for the
33 control carrier rate we estimate that these five genes contribute to the risk of epilepsy in
34 approximately 8% of familial NAFE cases. Our analyses showed that no individual gene was
35 significantly associated with epilepsy; however, known epilepsy genes achieved lower p -
36 values relative to the rest of the protein-coding genes ($p=5.8\times 10^{-8}$).

37 **INTERPRETATION**

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

45 **FUNDING**

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

47

INTRODUCTION

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

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

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

METHODS

69

70 **Participants**

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

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

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

94 **Procedures**

95 Sequencing was performed at the Institute for Genomic Medicine, Columbia University (New
96 York City, NY, USA). Samples were exome sequenced using the Agilent All Exon (50MB or
97 65MB; Agilent Technologies, Santa Clara, CA, USA) or the Nimblegen SeqCap EZ V2.0 or
98 3.0 Exome Enrichment kit (Roche NimbleGen, Madison, WI, USA) or whole genome
99 sequenced using HiSeq 2000 or 2500 (Illumina, San Diego, CA, USA) sequencers according
100 to standard protocols.

101 The sequence data from patients with epilepsy and controls were processed using the same
102 Institute for Genomic Medicine bioinformatics pipeline (appendix). We focused on 18,668
103 consensus coding sequence (CCDS; release 14) protein-coding genes. On average, at least
104 10-fold coverage was achieved for 95.8% (familial GGE), 96.8% (familial NAFE), 97.1%
105 (sporadic NAFE) and 95.6% (controls) of the 33.27 Mbps of the CCDS. For each protein-
106 coding site in the CCDS—inclusive of two base intronic extensions to accommodate
107 canonical splice variants—we determined the percentage of cases and controls that had ≥ 10 -
108 fold coverage at the site. To alleviate confounding due to differential coverage we used a site-
109 based pruning strategy similar to our previously described exon-pruning strategy.¹⁷ Individual
110 CCDS sites were excluded from analysis if the absolute difference in the percentage of the
111 cases compared to controls with adequate coverage of the site differed by greater than 5.19%
112 (familial GGE vs. controls), 5.14% (familial NAFE vs. controls) and 6.39% (sporadic NAFE
113 vs. controls) (appendix). Site-based pruning resulted in 8.9% (GGE), 8.3% (familial NAFE)
114 and 8.3% (sporadic NAFE) of the CCDS bases excluded from the respective analyses to
115 alleviate issues from differential coverage. Thus, all gene tests were performed on the pruned
116 CCDS where cases and controls had similar opportunity to call gene variants (appendix).

117

118 STATISTICAL ANALYSIS

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

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

137 For the primary analysis, functional annotation focused on single nucleotide substitution and
138 insertion or deletion variants annotated as having a loss-of-function, inframe insertion or
139 deletion, or a “probably damaging” missense effect by PolyPhen-2 (HumDiv).²⁰ Three
140 secondary analyses were performed to evaluate the contribution to epilepsy risk from: rare
141 loss-of-function variants with an internal and external population MAF up to 0.1%; rare non-
142 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 $p=8.9 \times 10^{-7}$, correcting for 18,668 CCDS genes studied across three models ($0.05/[3 \times 18668]$).
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).

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

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

173 **ROLE OF THE FUNDING SOURCE**

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

177

178 **RESULTS**

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

184

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

190 based on the disease-associated variant catalogues of ClinVar, the Online Mendelian
191 Inheritance in Man (OMIM), or the Human Gene Mutation Database (HGMD). All four
192 variants (two *SCN1A*, one *GABRG2*, and one *SCN1B*; appendix) were found among the
193 familial GGE cohort, an improbable enrichment given the expected proportion of 15.0%
194 ($p=5.1 \times 10^{-4}$, two-tailed exact binomial test). Through an evaluation of the scientific literature,
195 these four cases were confirmed as unrelated to those families reported in the literature.

196

197 While no single gene attained study-wide significance in the familial GGE analysis, three
198 known epilepsy genes (*KCNQ2*, *GABRG2*, and *SCN1A*), were among the top ten case-
199 enriched genes in the primary analysis (Figure 1). A hypergeometric test was run at each of
200 the gene ranks occupied by one of the 43 established epilepsy genes (appendix), and we
201 found that the enrichment was greatest at rank 151 whereby seven of the 43 known epilepsy
202 genes had been accounted for (hypergeometric $p=5.8 \times 10^{-8}$; appendix).

203

204 When we assessed enrichment among six biologically informed gene-sets, we found that the
205 familial GGE cohort had a significant enrichment of ultra-rare functional variation among 43
206 known dominant epilepsy genes ($p=9.1 \times 10^{-8}$, OR=2.3 [95% C.I. 1.7–3.2]; Table 2) and a
207 subset of 33 genes known to contribute to epileptic encephalopathy ($p=2.6 \times 10^{-7}$, OR=2.6
208 [95% C.I. 1.8–3.6]).³ We confirmed that the signal of enrichment for qualifying variants
209 among known epilepsy genes was consistently greater than the control rate across groupings
210 of the familial GGE cohort, reflecting the number of affected relatives (appendix). While they
211 did not achieve study-wide significance (defined as $p < 8.9 \times 10^{-7}$), we also investigated
212 qualifying variant enrichment among the fragile X mental retardation protein associated
213 genes,²² the genes encoding the NMDA receptor (NMDAR), and neuronal activity-regulated

214 cytoskeleton-associated protein, postsynaptic signalling complexes,²³ mouse seizure-
215 associated orthologs,²⁴ and ion channel protein-coding genes²⁵ (Table 2). None of these gene-
216 set tests reported enrichment of neutral variation.

217

218 Among the primary analysis of our familial NAFE cohort (figure 2A), *DEPDC5* achieved
219 study-wide significance (OR 8.1 [95% C.I. 3.6–18.3], $p=1.8 \times 10^{-7}$). *LGII* did not achieve
220 study-wide significance (OR 29.9 [95% C.I. 6.0–288.0], $p=1.4 \times 10^{-6}$). Established epilepsy
221 genes *PCDH19* (OR 22.4 [95% C.I. 4.0–226.4], $p=6.4 \times 10^{-5}$), *SCN1A* (OR 5.5 [95% C.I. 2.3–
222 12.9], $p=9.0 \times 10^{-5}$) and *GRIN2A* (OR 7.5 [95% C.I. 2.2–25.1], $p=5.3 \times 10^{-4}$) occupied the 3rd –
223 5th genome-wide ranks (appendix), but were not study-wide significant after correcting for
224 the 56,004 tests (Bonferroni corrected $p = 1$). A hypergeometric test indicated that it was
225 highly improbable for five of the 43 known dominant epilepsy genes to occupy the top five
226 positions of the primary analysis by chance ($p=5.7 \times 10^{-14}$) (appendix).

227

228 Of 74,272 qualifying variants identified in the primary analysis of 525 individuals with
229 familial NAFE and 3,877 controls, 9,092 (12.2%) of these were found among the familial
230 NAFE cases. Among the 74,272 qualifying variants, nine variants overlapped a codon of a
231 ClinVar, OMIM, or HGMD literature-reported pathogenic variant in a confirmed unrelated
232 family. All nine unique variants (three *DEPDC5*, three *PCDH19*, one *CHRN2*, one *GRIN2A*
233 and one *LGII* variant; appendix) were found among nine distinct NAFE cases of the
234 combined 4,402 unrelated samples used in the familial NAFE collapsing analysis, despite the
235 expected proportion being 12.2% (exact binomial test $p=6.2 \times 10^{-9}$).

236 The known dominant epilepsy gene-set (OR=3.6 [95% CI 2.7–4.9], $p=1.1 \times 10^{-17}$) and the
237 epileptic encephalopathy gene-set (OR=3.3 [95% CI 2.3–4.7], $p=5.0 \times 10^{-11}$) were study-wide
238 significantly enriched for qualifying variants among the primary analysis of familial NAFE
239 cases (Table 2). As observed in the familial GGE cases, the signal of enrichment for

240 qualifying variants among known epilepsy genes remained consistently greater than the
241 control rate across groupings of the familial NAFE cohort stratified by the number of affected
242 relatives (appendix). Presumably neutral variation was not significantly enriched among any
243 gene-set. Under the loss-of-function model, *DEPDC5* achieved study-wide significance
244 (OR=53.07, [95% C.I. 12.1–481.3], $p=9.6 \times 10^{-12}$), with 14 (2.7%) of familial NAFE cases
245 having a *DEPDC5* loss-of-function variant compared to only two (0.05%) controls. Focusing
246 solely on PolyPhen-2 ‘probably damaging’ missense *DEPDC5* qualifying variants showed
247 that they were non-significant for enrichment (3 [0.6%] of 525 cases vs. 12 [0.3%] of
248 3877 controls; OR=1.9 [95% C.I. 0.3–6.9], $p=0.41$; Figure 2B and appendix). Results from
249 the list of 43 known dominant epilepsy genes that achieved an uncorrected $p < 0.05$ in the
250 primary or loss-of-function models are listed in the appendix.

251

252 Sanger sequencing was used to validate a subset of qualifying variants found among 19
253 established and 13 candidate epilepsy genes (appendix). Our rate of Sanger validation was
254 97.0% (128/132) of the qualifying variants identified through the collapsing tests (appendix).
255 When available, we also Sanger sequenced qualifying variants among affected first-degree
256 relatives of index cases used in the collapsing analyses. We looked at six genes where we had
257 enough affected first-degree relatives to be sufficiently powered to achieve an uncorrected
258 $p < 0.05$ from a test of preferential segregation (appendix). Comparing to the expected rate of
259 50%, *SCN1A* (88.2% co-occurrence; $p=1.2 \times 10^{-3}$), *DEPDC5* (100% co-occurrence; $p=4.9 \times 10^{-4}$)
260 and *GRIN2A* (100% co-occurrence; $p=7.8 \times 10^{-3}$) had significant co-occurrence among
261 affected first-degree family members, after correcting for the six studied genes (adjusted
262 $\alpha=8.3 \times 10^{-3}$; appendix).

263

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

270

271 Comparing 662 sporadic NAFE cases to controls did not identify study-wide significant
272 genes across any of the three models (appendix). Of the five previously described familial
273 NAFE top ranked genes, we found that only *LGII* achieved an uncorrected p-value of less
274 than 0.05, (OR 8.8 [95% C.I. 1.0–105.7], p=0.025). None of the tested gene-sets were
275 significantly enriched with qualifying variants among sporadic NAFE cases (Table 2, Figure
276 3).

277

278

DISCUSSION

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

288 the example from *LGII*, 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 ($p < 8.9 \times 10^{-7}$)
291 for this known familial NAFE gene. Assuming the sampled rates for *LGII* case and control
292 carriers remain the same, we estimate that *LGII* 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 cohorts¹¹ 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

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

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

329 **RESEARCH IN CONTEXT**

330 **Evidence before this study**

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

341 **Added value of this study**

342 We used whole-exome sequencing on a large collection of two common epilepsy syndromes,
343 genetic generalized epilepsy (GGE) and non-acquired focal epilepsy (NAFE), to search for an
344 excess of ultra-rare deleterious qualifying variants, and compared the qualifying variant rates
345 found among cases to background rates estimated from sequenced controls. Among familial
346 index cases sampled from the common epilepsies, we found a significant excess of ultra-rare
347 deleterious variation within known epileptic encephalopathy genes. We also demonstrate that
348 the epilepsy risk signal observed in the known epilepsy genes is accounted for by the ultra-
349 rare class of variants that are absent among large reference control cohorts, such as ExAC and
350 EVS. Variants in known epilepsy genes that were predicted to be deleterious, but found at
351 very low frequencies among the population reference cohorts, showed no evidence of
352 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

366 **CONTRIBUTORS**

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383

384 **DECLARATION OF INTERESTS**

385 DBG reports personal fees from Pairnomix and EpiPM /Clarus outside the submitted work.
386 DA reports grants from NINDS during the conduct of the study. EA reports grants from
387 NINDS/NIH during the conduct of the study, and grants from Sunovion Pharmaceuticals Inc.
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390 reports grants from National Health and Medical Research Council and NINDS during the
391 conduct of the study; grants from UCB Pharma, Novartis Pharmaceuticals, Sanofi-Aventis,
392 Jansen Cilag, outside the submitted work; In addition, SFB has a patent for SCN1A testing
393 held by Bionomics Inc and licensed to various diagnostic companies. No financial return
394 although was a consultant to Bionomics and Athena diagnostics over 4 years ago; Patent
395 pending for DEPDC5 testing applied for by University of South Australia and University of
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398 OD served on the scientific advisory board of Paimomix during the conduct of the study. DD
399 reports grants from NIH/NINDS during the conduct of the study. MPE reports grants from
400 NIH/NINDS during the conduct of the study. EBG reports grants and personal fees from
401 Neuropace Inc outside the submitted work. TG reports grants from NIH (NS053998) during
402 the conduct of the study, and personal fees from AssureX Health, outside the submitted work;
403 also has a patent (PCT/US2006/045631) with royalties paid by AssureX Health. EHK served
404 on the Data Safety Board of GW Pharma, served on the Scientific Advisory Board of Atkins
405 Nutritionals, and reports grants from Nutricia outside the reported work. RK reports grants
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408 outside the submitted work. KP reports grants from NIH during the conduct of the study.
409 SPetrou reports equity from Paimomix outside the submitted work; a patent for the diagnosis
410 and treatment of epilepsy (WO2004/085674) issued to Bionomics Ltd, and a patent for
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412 gene(WO2006/133508) issued to Bionomics Ltd. SPetrov reports interests in Paimomix
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519 personalized therapy with memantine. *Annals of clinical and translational neurology* 2014; **1**(3): 190-
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527

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	

528 **Table 1.** Qualifying variant criteria in the four models.

529 [^]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
530 recurrent pathogenic variants that might be relevant to multiple cases. GGE = genetic generalized epilepsy. NAFE = non-acquired focal epilepsy. LoF = loss-of-function. MAF
531 = minor allele frequency. CCDS = consensus coding sequence

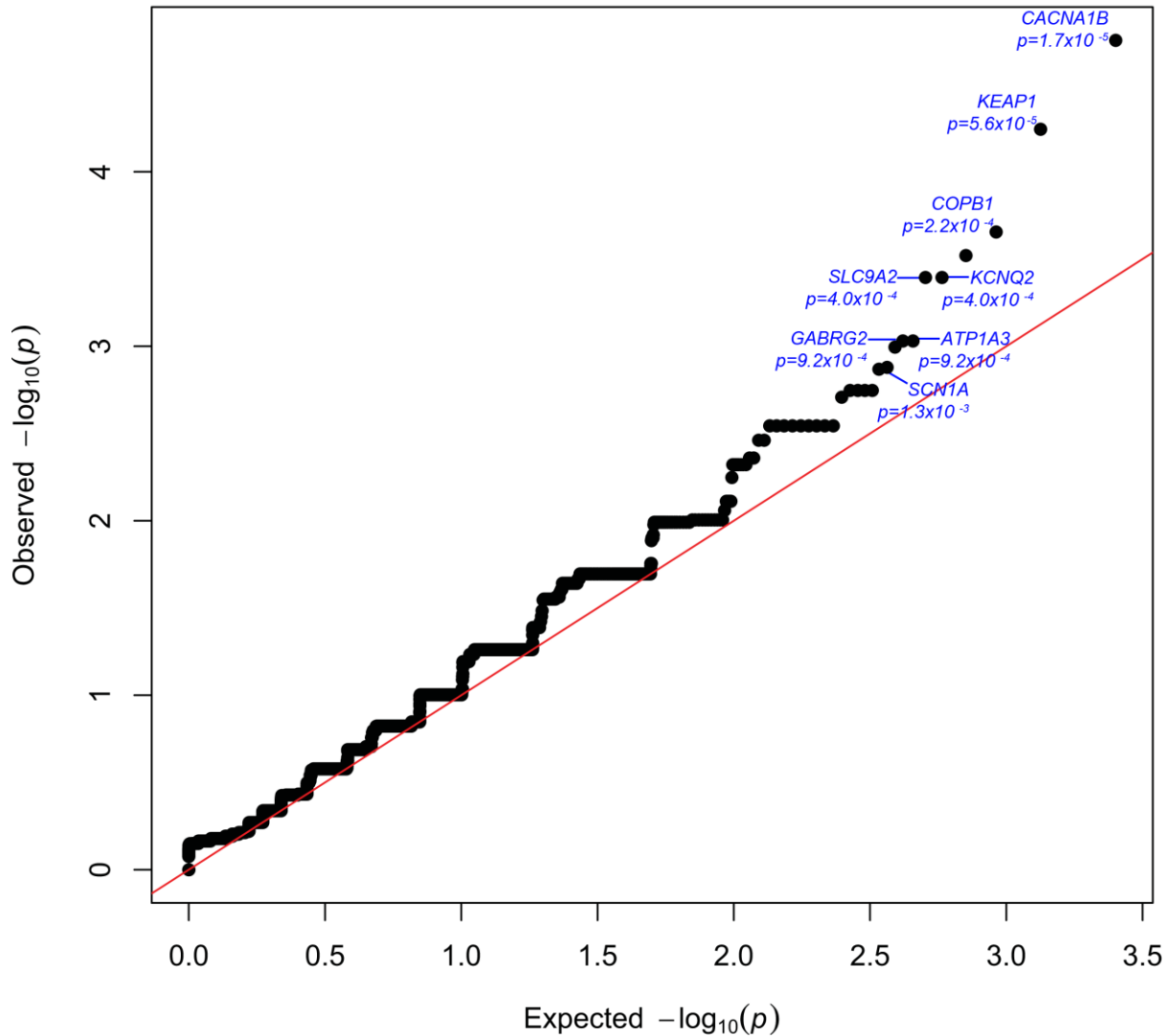
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Group	Gene set	Number of genes	Average qualifying variants ^a	Qualifying variants enrichment p-value (Odds Ratio [95% CI])	Neutral variation enrichment p-value	Enrichment after removing the 43 epilepsy genes p-value
Familial GGE	Known	43	0.052	$p = 9.1 \times 10^{-8}$ (OR=2.3 [95% CI 1.7 - 3.2])	$p = 0.86$	N/A
	Known (EE)	33	0.037	$p = 2.6 \times 10^{-7}$ (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	$p = 1.1 \times 10^{-17}$ (OR=3.6 [95% CI 2.7 - 4.9])	$p = 0.87$	N/A
	Known (EE)	33	0.037	$p = 5.0 \times 10^{-11}$ (OR=3.3 [95% CI 2.3 - 4.7])	$p = 0.65$	N/A
	Ion Channel	209	0.264	$p = 1.9 \times 10^{-4}$ (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])	$p = 0.33$	$p = 0.33$

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

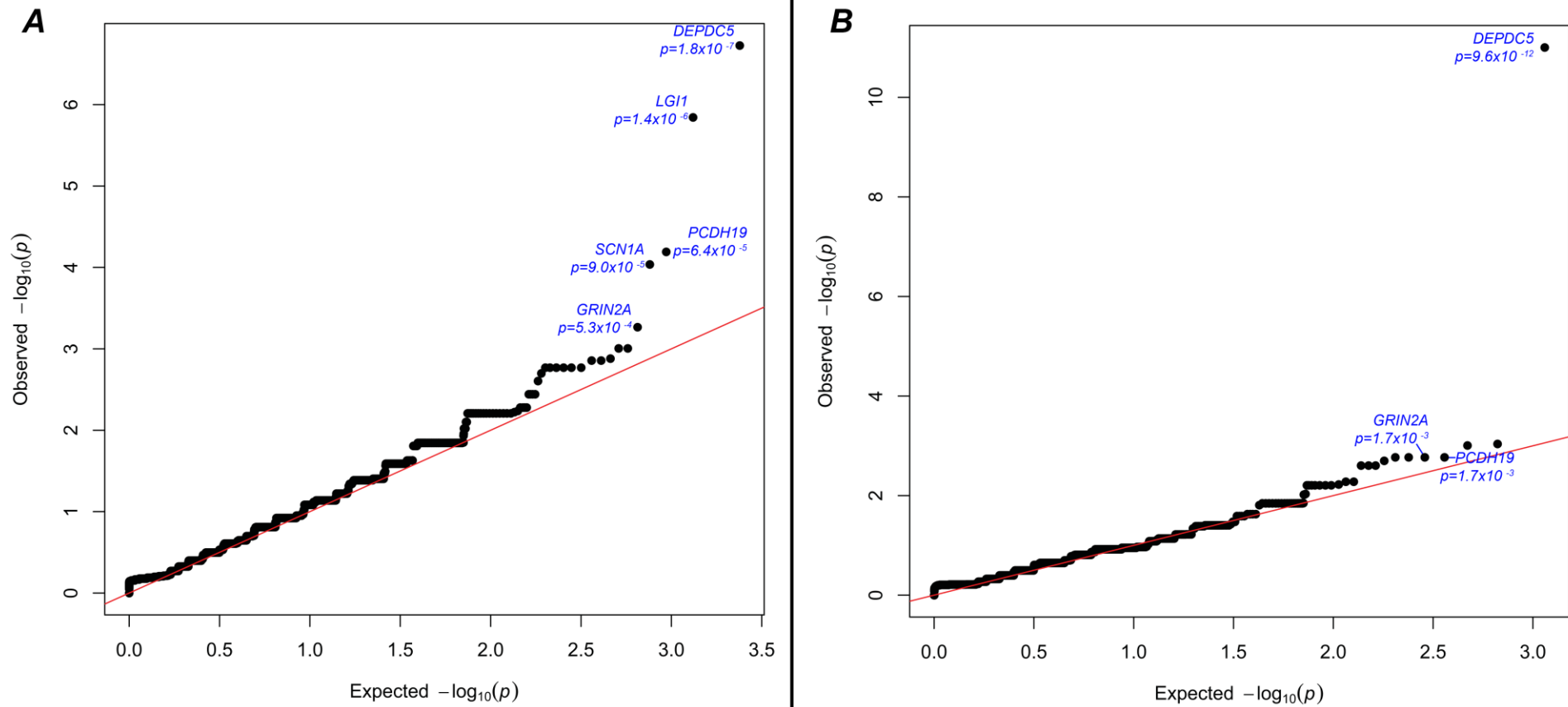
540 channels.²⁵ **FMRP** = fragile X mental retardation protein associated genes.²² **NMDAR & ARC** = NMDA
541 receptor and neuronal activity-regulated cytoskeleton-associated protein synaptic transmission genes.²³ **MGI**
542 **Seizure** = mouse orthologs linked with seizure phenotypes in the Mouse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

544 **Figure 1: Familial GGE primary model analysis.** 15,515 genes had at least one case or control
545 carrier (table 1). Qualifying variants were defined as a minor allele frequency <0.05% in internal case
546 and control, and absent among external reference cohorts. Variants are annotated as loss-of-function,
547 inframe insertions or deletions, or missense predicted to be “probably damaging” by PolyPhen-2
548 (HumDiv). No gene achieved study-wide significance (adjusted $\alpha < 0.05/[18668 * 3] = 8.9 \times 10^{-7}$).



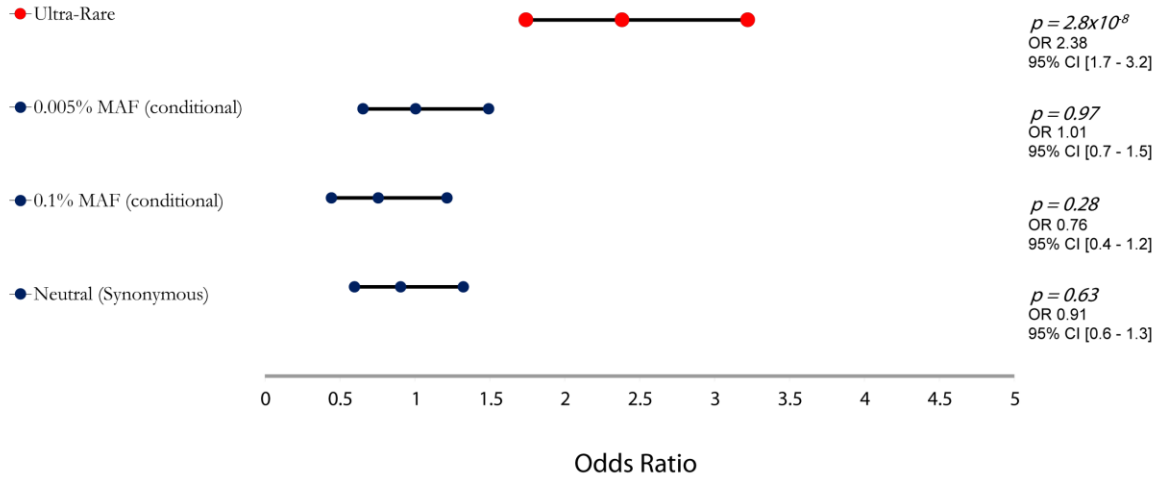
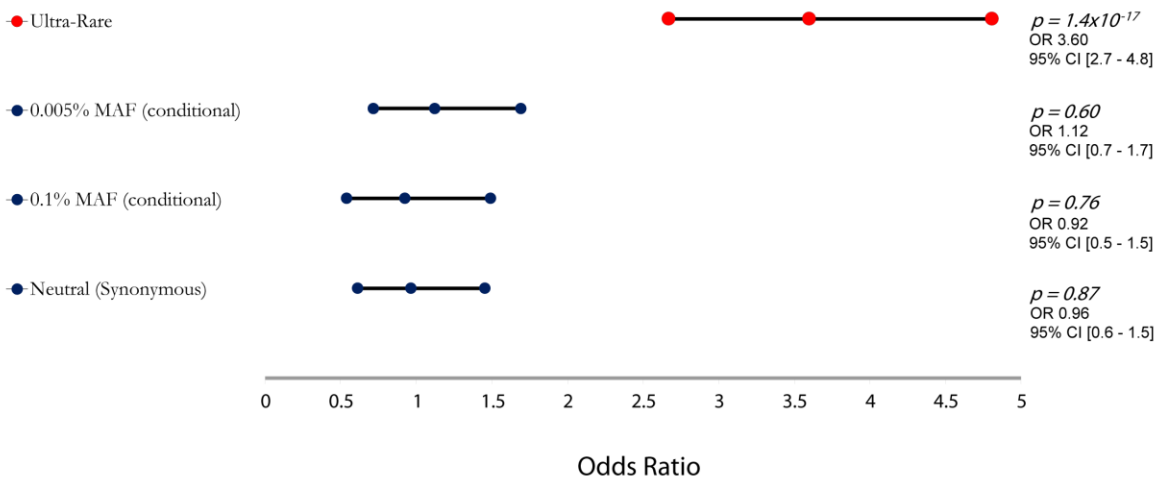
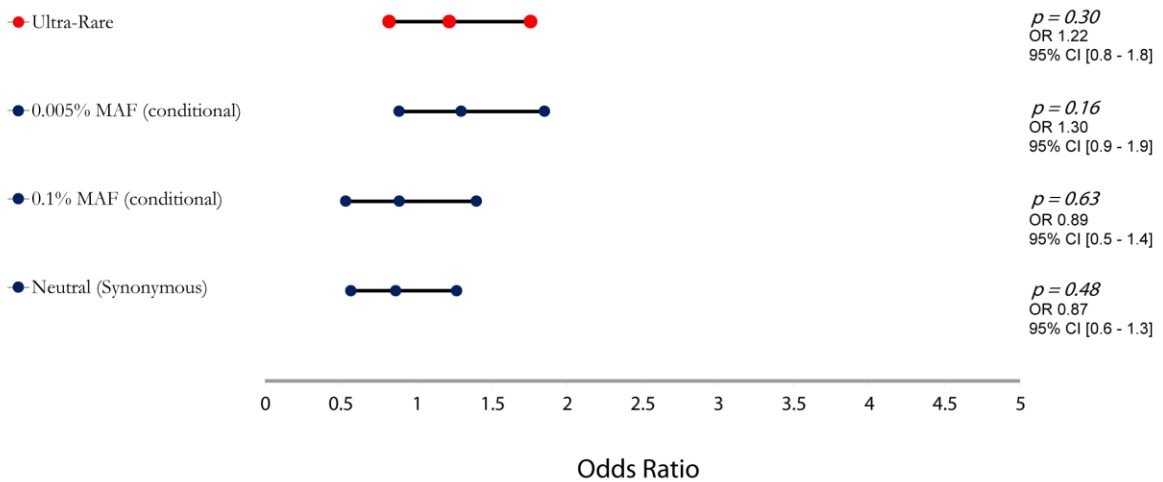
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551 **Figure 2: Familial NAFE primary model analysis.** (A) 15,438 genes had at least one case or control carrier (table 1). Qualifying variants have a minor allele
 552 frequency <0.05% in internal case and control, and are absent among external reference cohorts. Variants are annotated as loss-of-function, inframe insertions or
 553 deletions, or missense predicted to be “probably damaging” by PolyPhen-2 (HumDiv). Only *DEPDC5*, achieved study-wide significance (adjusted $\alpha <$
 554 $0.05/[18668 * 3] = 8.9 \times 10^{-7}$). (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
 555 annotated as loss-of-function effects. Only *DEPDC5* achieved study-wide significance.



556

557 **Figure 3: Enrichment of qualifying variants among 43 known epilepsy genes across increasing**
558 **minor allele frequency bins.** The ultra-rare variation bin reflects qualifying variants from the
559 primary analyses. The 0.005% MAF (conditional) bin represents qualifying variants with a MAF
560 greater than 0% but no greater than 0.005% in ExAC. The 0.1% MAF (conditional) bin represents
561 qualifying variants with a MAF greater than 0.005% but no greater than 0.1% in ExAC. The neutral
562 (synonymous)bin represents ultra-rare putatively neutral variants across the 43 epilepsy genes.
563 Multivariate conditional analyses for the **(A)** familial GGE population **(B)** familial NAFE population
564 **(C)** sporadic NAFE
565

A**B****C**

CONSORTIA

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