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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 (GGE: OR 2.3 [95% CI 1.7–3.2]; p=9.1x10^{-8}) (NAFE: OR 3.6 [95% CI

29 2.7–4.9]; p=1.1x10^{-17}). Comparing an additional collection of 662 individuals with sporadic

30 NAFE to controls did not identify study-wide significant signals. For the familial NAFE

31 cases, we found that five previously known epilepsy genes ranked as the top five genes

32 enriched for ultra-rare deleterious variation. After accounting for the control carrier rate we

33 estimate that these five genes contribute to the risk of epilepsy in approximately 8% of

34 familial NAFE cases. While no individual gene showed study-wide significance in the

35 familial GGE analyses, known epilepsy genes showed a significant excess (p=5.8x10^{-8}) of p-

36 values that were lower than expected from a random sampling of genes.

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**

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

6

0.1%; and a presumed neutral model that imposed similar MAF thresholds as our primary

analysis, but focused specifically on protein-coding variants predicted to have a synonymous

effect. The purpose of the presumed neutral model was to further confirm that no cryptic

factors might be increasing qualifying variant calling among one of the groups.

For each of the four models, we tested the complete list of 18,668 CCDS genes. For each

gene, an indicator variable (1/0 states) was assigned to each individual based on the presence

of at least one qualifying variant in the gene (state 1) or no qualifying variants in that gene

(state 0). We used a two-tailed Fisher's exact test to identify genes where there was a

significant enrichment of qualifying variants in the case or control group. To control for the

type-I error rate within each epilepsy phenotype, we defined study-wide significance as

$p=8.9\times10^{-7}$, correcting for 18,668 CCDS genes studied across three models (0.05/[3x18668]).

We did not correct for the neutral control model.

All collapsing analyses were performed using an in-house package, Analysis Tool for

Annotated Variants (ATAV). Binomial tests were used to evaluate whether there was an

enrichment of previously reported pathogenic variants among the case collection of

qualifying variants. Hypergeometric tests were performed to assess whether among the

collapsing analysis results the known epilepsy genes preferentially achieved lower p-values

relative to the rest of the genome. Cochran-Mantel-Haenszel tests were adopted to combine

the results of the gender stratified sex chromosome collapsing analyses.

We also used the primary analysis results from each of the patient groups to assess

enrichment among six biologically informed gene-sets that were chosen and described in our

earlier studies of the epileptic encephalopathies,[5, 21] including a list of 43 established

dominant epilepsy genes (appendix).[3] To account for background variation in gene-set tests

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 <div align="center">**RESULTS**</div>

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=5.1x10^{-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=5.8x10^{-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=9.1x10^{-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=2.6x10^{-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< 8.9x10^{-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 genes[25] (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=1.8×10^{-7}). *LGI1* did not achieve

220   study-wide significance (OR 29.9 [95% C.I. 6.0–288.0], p=1.4×10^{-6}). Established epilepsy

221   genes *PCDH19* (OR 22.4 [95% C.I. 4.0–226.4], p=6.4×10^{-5}), *SCN1A* (OR 5.5 [95% C.I. 2.3–

222   12.9], p=9.0×10^{-5}) and *GRIN2A* (OR 7.5 [95% C.I. 2.2–25.1], p=5.3×10^{-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*=5.7×10^{-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 *CHRNB2,* one *GRIN2A*

233   and one *LGI1* 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=6.2×10^{-9}).

236   The known dominant epilepsy gene-set (OR=3.6 [95% CI 2.7–4.9], *p*=1.1×10^{-17}) and the

237   epileptic encephalopathy gene-set (OR=3.3 [95% CI 2.3–4.7], *p*=5.0×10^{-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=9.6x10^{-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 3877controls; 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=1.2x10^{-3}), *DEPDC5* (100% co-occurrence; p=4.9x10^{-}

260 ^{4}) and *GRIN2A* (100% co-occurrence; p=7.8x10^{-3}) had significant co-occurrence among

261 affected first-degree family members, after correcting for the six studied genes (adjusted

262 α=8.3x10^{-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 *LGI1* 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, LGI1, PCDH19, SCN1,A* 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 *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 ($p < 8.9 \times 10^{-7}$)

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

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.

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.

Implications of all the available evidence

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

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DECLARATION OF INTERESTS

428   ACKNOWLEDGEMENTS

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Cohort	Model	Internal MAF(%)	External MAF(%)	Variant Effects	# Genes with >0 qualifying variant(s)
Familial GGE	Primary^	0.05%	0%	LoF inframe insertions or deletions PolyPhen-2 (HumDiv) "probably" damaging	15,515
	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
	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
	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*

recurrent pathogenic variants that might be relevant to multiple cases. GGE = genetic generalized epilepsy. NAFE = non-acquired f

*= 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*	**$p = 9.1 \times 10^{-8}$ (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*

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 $\alpha = 8.9 \times 10^{-7}$. 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

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 $\alpha < 0.05/[18668 * 3] = 8.9 \times 10^{-7}$).
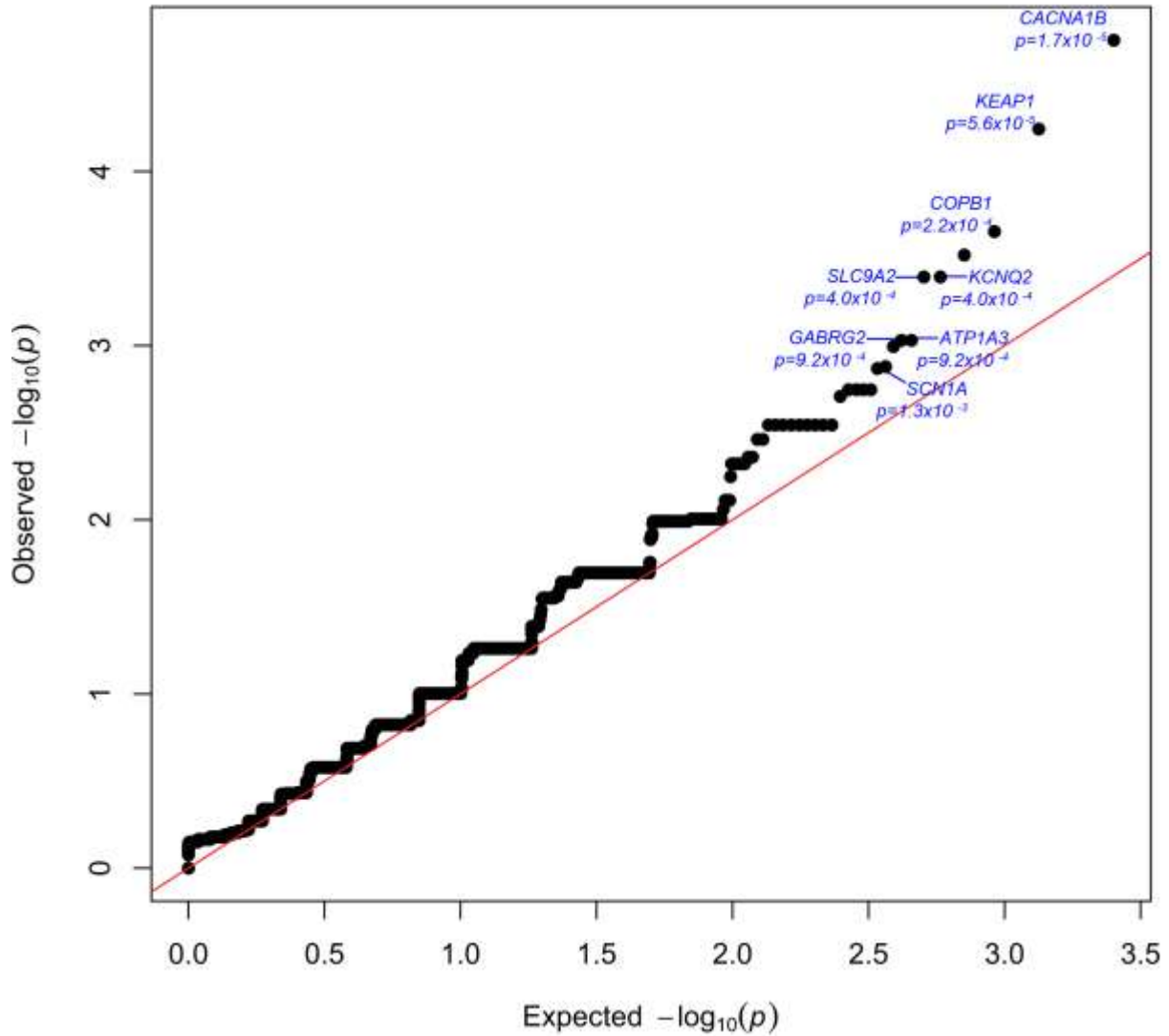
549
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551 **Figure 2: Familial NAFE primary model analysis. (A)** 15,438 genes had at least one case or control carrier (table 1). Q

552 frequency <0.05% in internal case and control, and are absent among external reference cohorts. Variants are annotated a

553 deletions, or missense predicted to be "probably damaging" by PolyPhen-2 (HumDiv). Only *DEPDC5*, achieved study-w

554 0.05/[18668 * 3] = 8.9x10^{-7}). **(B)** 10,601 genes had at least one case or control carrier (table 1). Qualifying variants are v

555 annotated as loss-of-function effects. Only *DEPDC5* achieved study-wide significance.

556
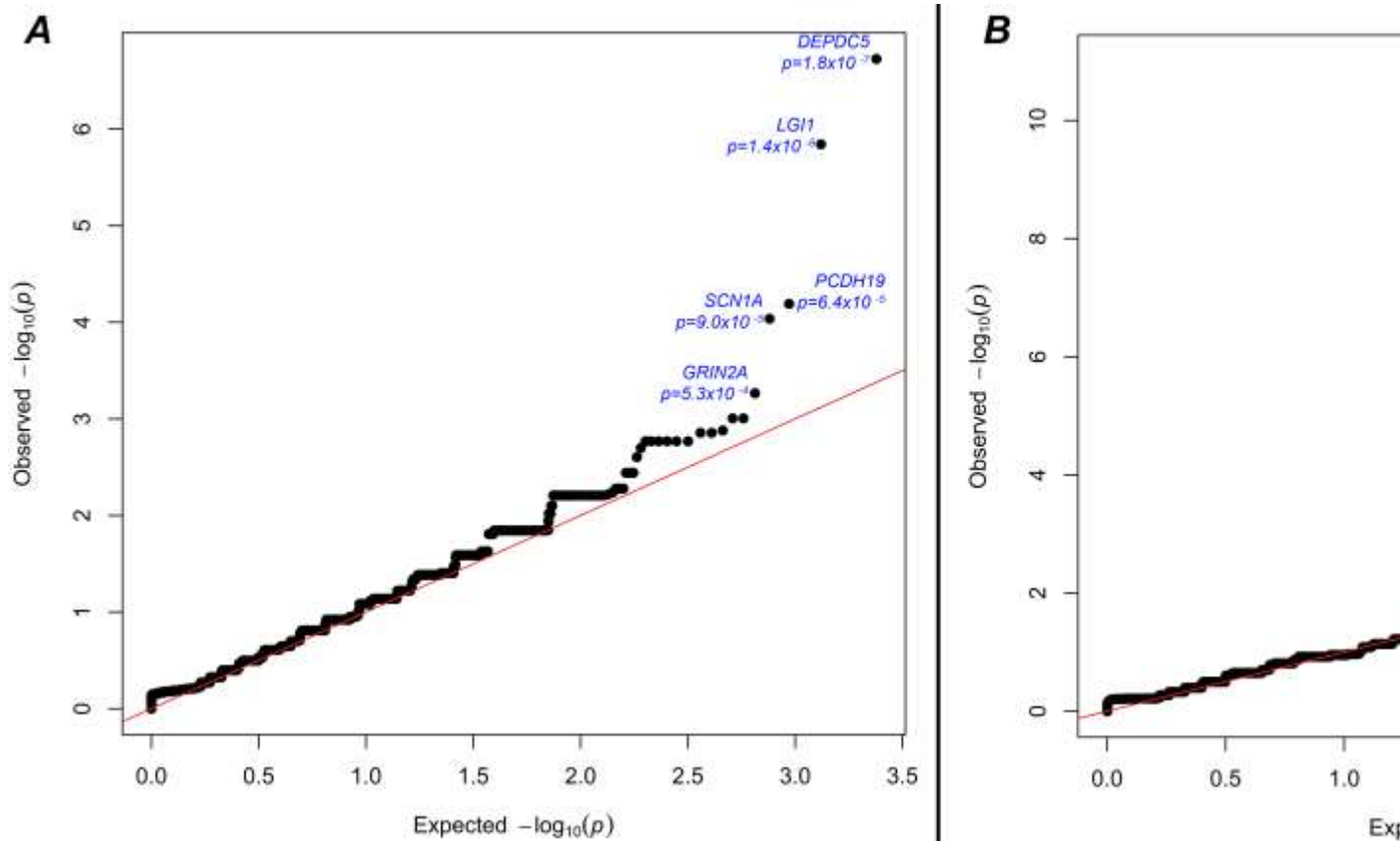
557 **Figure 3: Enrichment of qualifying variants among 43 known epilepsy genes across increasing**

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

- Ultra-Rare — $p = 2.8 \times 10^{8}$
OR 2.38
95% CI [1.7 - 3.2]

- 0.005% MAF (conditional) — $p = 0.97$
OR 1.01
95% CI [0.7 - 1.5]

- 0.1% MAF (conditional) — $p = 0.28$
OR 0.76
95% CI [0.4 - 1.2]

- Neutral (Synonymous) — $p = 0.63$
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 — $p = 1.4 \times 10^{-17}$
OR 3.60
95% CI [2.7 - 4.8]

- 0.005% MAF (conditional) — $p = 0.60$
OR 1.12
95% CI [0.7 - 1.7]

- 0.1% MAF (conditional) — $p = 0.76$
OR 0.92
95% CI [0.5 - 1.5]

- Neutral (Synonymous) — $p = 0.87$
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 — $p = 0.30$
OR 1.22
95% CI [0.8 - 1.8]

- 0.005% MAF (conditional) — $p = 0.16$
OR 1.30
95% CI [0.9 - 1.9]

- 0.1% MAF (conditional) — $p = 0.63$
OR 0.89
95% CI [0.5 - 1.4]

- Neutral (Synonymous) — $p = 0.48$
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

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