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Phenotypic Analysis of 303 Multiplex Families with Common Epilepsies

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PHENOTYPES IN 303 EPILEPSY FAMILIES

Gene identification in epilepsy has mainly been limited to large families segregating genes of major effect and de novo mutations in epileptic encephalopathies. Many families that present with common non-acquired focal epilepsies and genetic generalized epilepsies remain unexplained. We assembled a cohort of ‘genetically enriched’ common epilepsies by collecting and phenotyping families containing multiple individuals with unprovoked seizures. We aimed to determine if specific clinical epilepsy features aggregate within families, and whether this segregation of phenotypes may constitute distinct ‘familial syndromes’, that could inform genomic analyses. Families with three or more individuals with unprovoked seizures were studied across multiple international centers. Affected individuals were phenotyped and classified according to specific electro-clinical syndromes. Families were categorized based on syndromic groupings of affected family members, examined for pedigree structure and phenotypic patterns, and, where possible, assigned specific familial epilepsy syndromes. A total of 303 families were assembled and analyzed, comprising 1120 affected phenotyped individuals. Of the 303 families, 117 exclusively segregated generalized epilepsy, 62 focal epilepsy, and 22 were classified as genetic epilepsy with febrile seizures plus. Over one third (102 families) were observed to have mixed epilepsy phenotypes: 78 had both generalized and focal epilepsy features within the same individual (n=39), or within first or second degree relatives (n=39). Among the genetic generalized epilepsy families, absence epilepsies were found to cluster within families independently of juvenile myoclonic epilepsy, and significantly more females were affected than males. Of the 62 familial focal epilepsy families, two previously undescribed familial focal syndrome patterns were evident: 15 families had posterior quadrant epilepsies, including seven with occipito-temporal localization and seven with temporo-parietal foci, and four families displayed familial focal epilepsy of childhood with multiple affected siblings that was suggestive of recessive inheritance. The findings suggest (1) specific patterns of syndromic familial aggregation occur, including newly recognized forms of familial focal epilepsy, (2) although syndrome-specificity usually occurs in multiplex families, the one third of families with features of both focal and generalized epilepsy is suggestive of shared genetic determinants, (3) patterns of features observed across families including pedigree structure, sex, and age of onset may hold clues for future gene identification. Such detailed phenotypic information will be invaluable in the conditioning and interpretation of forthcoming sequencing data to understand the genetic architecture and inter-relationships of the common epilepsy syndromes.

**Keywords:** Epilepsy, multiplex families, phenotype, genetics

**Abbreviations:** CAE=Childhood absence epilepsy, CECTS=Childhood epilepsy with centrotemporal spikes, FS=Febrile seizures, GEFS+=Genetic epilepsy with febrile seizures plus, GGE=Genetic generalized epilepsy, IPOE=Idiopathic photosensitive occipital epilepsy,
PHENOTYPES IN 303 EPILEPSY FAMILIES
JME=Juvenile myoclonic epilepsy, MAE=Epilepsy with myoclonic-atonic seizures, NAFE=Non-acquired focal epilepsy, TLE=Temporal lobe epilepsy
PHENOTYPES IN 303 EPILEPSY FAMILIES

Introduction

Much of the initial success in molecular genetics of epilepsy involved careful phenotyping of large families, typically with 10 or more affected individuals, followed by linkage analysis and gene identification by the positional candidate approach. Genes for relatively mild forms of generalized and focal epilepsies were discovered in large dominant families. Notable successes included genes identified in families with autosomal dominant nocturnal frontal lobe epilepsy (Steinlein et al., 1995), genetic epilepsy with febrile seizures plus (GEFS+) (Wallace et al., 1998, Escayg et al., 2000), autosomal dominant partial epilepsy with auditory features (Kalachikov et al., 2002), familial focal epilepsy with variable foci (Dibbens et al., 2013), and generalized epilepsy and paroxysmal exercise-induced dyskinesia (Suls et al., 2008, Mullen et al., 2010). More recently, de novo mutagenesis has emerged as the major genetic mechanism in epileptic encephalopathies and rapid progress has been facilitated by whole exome sequencing (Claes et al., 2001, Epi4K Consortium et al., 2013, Euro Epinomics et al., 2014).

In the majority of cases with common epilepsies, however, inheritance appears complex (Ottman, 2005, Helbig et al., 2008, Epi4K Consortium, 2012) and molecular genetic advances have been modest. Most affected individuals have no known family history or just a few affected family members. Population genetic and twin studies suggest that multiple genes may be contributing to each case, with evidence of both shared and distinct genetic influences on major syndrome type (generalized versus focal epilepsy) (Berkovic et al., 1998, Ottman et al., 1998, Kjeldsen et al., 2003, Winawer et al., 2003a, Vadlamudi et al., 2004b, Peljto et al., 2014, Vadlamudi et al., 2014), although the number of genes and the extent to which they raise risk (effect size) are unknown (Ottman et al., 1997, Berkovic et al., 1998). Our recent analysis of exome sequencing in unrelated individuals with a family history of epilepsy shows an increased burden of ultra-rare variants among the currently known epilepsy genes (Epi4K Consortium and Epilepsy Phenome/Genome Project, 2017). Despite this knowledge, the overall genetic architecture of the common epilepsies remains unclear. This is a general problem in the field of common complex diseases. It may be that in-depth phenotyping, which can be done in genetic epilepsies to a greater extent than many other complex traits, may provide critical clues to aid genetic analyses.

To this end, a major aim of Epi4K (Epi4K Consortium, 2012) was to assemble and perform detailed phenotyping of a large collection of multiplex families with common epilepsies to inform interpretation of genotypes from next generation sequencing. Here, we analyze the clinical patterns observed in 303 systematically phenotyped families with three or more individuals with unprovoked seizures of unknown cause. We asked 1) Within this ‘genetically enriched’ cohort, do families clearly separate into generalized, focal or GEFS+, or do we observe many ‘mixed; families?; 2) Do
PHENOTYPES IN 303 EPILEPSY FAMILIES

syndromes of generalized epilepsy (absence epilepsies, juvenile myoclonic epilepsy and rarer syndromes) segregate separately?; 3) Within focal epilepsies, are new familial syndromes identifiable beyond the well-known familial frontal and familial temporal syndromes?; and 4) Does this data set hold any clues to conditioning genetic analyses in terms of sex, age of onset, pedigree structure to assist the interpretation of forthcoming genomic data?
Methods

Ascertainment of families

Families were ascertained from cohorts of the University of Melbourne, Columbia University (New York), University of Montreal, Swansea University, The Royal College of Surgeons, Ireland and the Epilepsy Phenome/Genome Project (EPGP Collaborative, 2013). Families were recruited from Australia (n=96), Canada (Quebec; n=25), Ireland (n=8), Israel (n=52), New Zealand (n=16), USA (n=80), and Wales (UK; n=26).

The Institutional Review Boards of the participating institutions approved the study protocol. Informed consent was obtained from all participants, or from parents or guardians of minors or individuals with intellectual disability.

Inclusion criteria for multiplex families

1. Three or more affected individuals with unprovoked seizures (single or multiple) of unknown cause (‘non-acquired’) where DNA was available.

2. Two or more of the affected individuals in the family classifiable by broad epilepsy syndrome (generalized, focal, or FS+). Individuals who fulfilled criteria for more than one broad syndrome were eligible. Families could contain other affected individuals with unprovoked seizures who were not classifiable by broad epilepsy syndrome.

Moderate or severe intellectual disability was an exclusion criterion as these cases are more likely to have a discrete cause (e.g. chromosomal deletion, undetected brain abnormality) rather than be related to the familial epilepsy. We phenotyped but excluded from further analyses individuals with: single or multiple febrile seizures (FS) only (who did not have FS+), provoked (acute symptomatic) seizures, or unprovoked seizures with an identified antecedent cause (e.g., stroke, severe traumatic brain injury, epileptogenic lesions, previously identified genetic cause). Hippocampal sclerosis was not an exclusionary criterion. Families were not required to have had prior genetic testing, however most had had clinically indicated genes examined where relevant (e.g. SLC2A1, SCN1A).

Classification of individuals

Clinical information was assessed on all available affected family members including, where available, detailed clinical interviews, eye-witness accounts of seizures, and medical records including EEG studies, video-EEG monitoring and neuroimaging studies. All individuals with seizures were phenotyped and classified into a broad epilepsy classification of generalized, focal, FS+, unclassified or mixed epilepsy, based on seizure type and EEG findings. Where possible, all
PHENOTYPES IN 303 EPILEPSY FAMILIES
affected individuals were then classified based upon EEG findings, seizure semiology and
developmental history according to specific electro-clinical syndromes as defined by the International
League Against Epilepsy publications (ILAE, 1985, 1989, Berg et al., 2010, ILAE Diagnostic

A diagnosis of generalized epilepsy was made in cases with absence, myoclonic, or tonic-
clonic seizures with an EEG showing generalized spike and wave discharges. In cases where EEG
records were unavailable or non-diagnostic, cases with a clinically persuasive history of myoclonic
seizures or typical absences were accepted. Non-acquired focal epilepsy (NAFE) required clinical
seizures consistent with a focal onset and at least one of the following: EEG showing interictal focal
epileptiform discharges, a focal seizure recorded on EEG, or clear history of focal onset of seizures
based on semiology if the EEG was normal. Cases were diagnosed as ‘mixed’ where there were
clinical or EEG features consistent with both generalized and focal epilepsy. Earlier research has
indicated idiopathic photosensitive occipital epilepsy (IPOE) often co-occurs with JME and could be
regarded as a form of genetic generalized epilepsy (GGE) when presenting with generalized epilepsy
features (Taylor et al., 2004, Taylor et al., 2013). Here we classified individuals with IPOE based on
presenting semiology and EEG evidence: focal epilepsy alone or mixed epilepsy where individuals
had generalized seizures and/or generalized epileptiform discharges in addition to focal seizures.
Cases with tonic-clonic seizures or non-convulsive seizures lacking clear semiology who did not have
an epileptiform EEG were categorized as unclassified epilepsy.

A diagnosis of FS+ required at least one of the following: (1) febrile seizures (with a
documented illness or temperature over 38°C) before six months, (2) febrile seizures extending
beyond six years, OR (3) febrile seizures within six months to six years plus afebrile tonic-clonic
seizures beginning before ten years of age (Scheffer and Berkovic, 1997, Singh et al., 1999).
Individuals were classified as FS+ and an additional epilepsy syndrome only if the presentation of
classical FS+ was followed by a period of seizure freedom before onset of later epilepsy, or in the
context of a well-defined electro-clinical syndrome with febrile seizures outside the six-month to six-
year timeframe. Individuals with a history of a single unprovoked seizure without an epileptiform
EEG abnormality were classified as an isolated seizure. Individuals with FS alone were considered
in the identification of GEFS+ families (see classification of families), but not counted in familial
analyses.

Consistency of diagnostic classification between sites was monitored during data collection by
review of data entry forms of at least one in five families by two other sites. Discordances in
phenotypic designations occurred infrequently, and were resolved by consensus discussion.
PHENOTYPES IN 303 EPILEPSY FAMILIES

Classification of families

Pedigrees of the 303 families were categorized according to four broad familial epilepsy categories, and then examined for patterns of phenotypic segregation and familial epilepsy syndromes. Broad familial epilepsy categories included GGE and familial focal epilepsy (where all eligible classified cases in the family had GGE or NAFE respectively), GEFS+ and ‘mixed’ families. Families were classified as having a specific familial epilepsy syndrome (e.g., familial temporal lobe epilepsy) if they contained two or more family members classified with the specified phenotype; other family members may have less well-defined (e.g. non-localized focal epilepsy) but not discordant phenotypes (e.g., TLE and CECTS).

GEFS+ is a familial syndrome that encompasses a spectrum of phenotypes; the core GEFS+ phenotype is FS+ (Scheffer and Berkovic, 1997). Some large families with GEFS+ and known pathogenic mutations contain rare individuals with well-defined electroclinical syndromes of GGE (especially CAE) or NAFE (especially TLE). Identification of GEFS+ is difficult in smaller families containing few individuals with FS+ and other family members with GGE or NAFE. Here we defined GEFS+ families as those that included at least one individual with FS+ and at least one other individual with either febrile seizures or FS+, even if there were additional individuals with GGE or NAFE.

Mixed families demonstrated clear evidence of both GGE and NAFE epilepsy syndromes in separate individuals or in one person. Families were also categorized as mixed if they contained only a single individual with FS+ and no other individuals with febrile seizures.

Pedigree configuration

We recorded the number of meiotic events between the most distantly related affected individuals as a measure of degree of relatedness of affected individuals within families. To investigate the segregation within families, pedigrees were also classified as ‘horizontal’ inheritance if only siblings or siblings and a cousin were affected, or ‘vertical’ inheritance with three or more sequential generations affected with epilepsy. Consanguinity and bilinearity were also recorded.

Statistical analyses

We constructed statistical tests to investigate whether variables of interest (including age of onset, sex, number of affected individuals per family, and number of meiotic events per family) varied significantly across different familial epilepsy categories. For analyses involving independent
observations, we employed either standard regression procedures (for continuous or binary outcomes) or Kruskal-Wallis tests (for ordinal categorical outcomes such as number of affected individuals per family). For analyses involving dependent observations (such as data from multiple correlated relatives within the same family), we performed analyses using generalized estimating equations (GEE) that accounted for within-family correlation. We also examined whether particular clinical syndromes clustered within families using Krippendorff’s alpha coefficient (Krippendorff, 1980). We performed 15 tests in total and corrected for multiple testing by applying a Bonferroni correction. We report these Bonferroni-corrected p-values in the text. We performed all analyses using the R programming language.
PHENOTYPES IN 303 EPILEPSY FAMILIES

Results

A total of 303 families meeting inclusion criteria were ascertained and phenotyped by the collaborating sites. These families comprised 1120 individuals affected with epilepsy of unknown cause (Table 1) and included an additional 106 with FS alone. Families contained a mean of 3.7 (range 3-8) affected individuals: 170 with three affected, 87 with four affected, 26 with five affected, and 20 with six or more affected individuals. Relatedness of affected family members ranged from all first-degree relatives (e.g. parent/children or siblings) in 120 families, to six meiotic events between the most distantly related individuals in three families (Table 2).

Distribution of familial syndromes and ‘mixed’ families

There were 117 families where all classifiable affected individuals had genetic generalized epilepsy, 62 families where all had focal epilepsy, 22 families had GEFS+ phenotypes, and 102 families had ‘mixed’ syndromes.

The 102 ‘mixed’ families (387 individuals) are of particular interest. Figure 1 shows the minimum number of meioses between family members with generalized and focal seizure semiology. The majority of these families (76%) had focal and generalized epilepsy diagnoses occurring either within the same individual (39 families; Fig. 2A) or closely related family members (1-2 meiotic events between individuals (39 families; Fig. 2B).

In 21 (21%) of the ‘mixed’ families, individuals with focal and generalized epilepsies were more widely dispersed, with three or more meiotic events between family members with different phenotypes (Fig. 2C). Here, one form of epilepsy (generalized or focal) often clustered in one branch with another phenotype occurring in more distant relatives. This suggests the other phenotype may be an ‘incidental’ occurrence, although the possibility of shared genetic determinants cannot be excluded. Five families showed bilineal family history of epilepsy, with generalized epilepsy in one branch and focal epilepsy in another.

Finally, three families had one individual with FS+ and others with either generalized or focal epilepsies. As there were no other family members with seizures associated with fever, the families did not meet our criteria for GEFS+.

The syndromes of generalized and focal epilepsy that co-occurred in the mixed families were heterogeneous, but some patterns were noted. Twenty individuals in 13 families had IPOE with both focal and generalized features (see Methods). Nine of these families had only generalized syndromes (especially juvenile myoclonic epilepsy) in addition to IPOE, whereas the remaining four families
had other focal and generalized epilepsies in addition to IPOE. Two of these families were categorized as mixed epilepsy based upon a single individual with IPOE. An additional individual had photosensitive occipital seizures without generalized seizures or epileptiform discharges on EEG; affected relatives had generalized epilepsy or isolated seizures.

Thirteen mixed families had generalized epilepsies (usually absence epilepsies) and a single individual with a self-limited focal epilepsy of childhood, either CECTS or Panayiotopoulos syndrome (e.g., Fig. 2B).

Juvenile myoclonic epilepsy co-occurred with a range of focal epilepsies in addition to IPOE (nine families described above), including temporal lobe epilepsy (six families) and posterior quadrant epilepsies (four families). Finally, absence and temporal lobe epilepsies, the two most common syndromes in the dataset (Table 1), co-occurred in 15 families.

** Syndromes in genetic generalized epilepsy families **

These 117 families comprised 338 individuals with a range of generalized epilepsy syndromes (Table 1) and 79 individuals in whom an epilepsy syndrome could not be specified.

We observed 42 cases (12%) with early-onset absence epilepsy, defined as onset under age four years, which until recently was regarded as rare. Sixteen individuals had ‘severe’ generalized epilepsy syndromes, arguably outside the spectrum of classical GGEs, including epilepsy with eyelid myoclonias (n=5), myoclonic absence epilepsy (n=4), epilepsy with myoclonic-ataxic seizures (MAE; n=6) and Lennox-Gastaut syndrome (n=1). The individual with Lennox-Gastaut syndrome did not meet inclusion criteria for the study; three other affected family members fulfilled inclusion criteria and were used for categorization.

We classified families into familial generalized syndromes based on comprising at least two family members with the same electro-clinical syndrome; other family members could have less well-defined syndromes (e.g. GGE unspecified or epilepsy with generalized tonic-clonic seizures alone), but not discordant phenotypes (Table 3). Amongst the 117 families, 48 (41%) had familial absence epilepsies (early-onset, childhood or juvenile) and 15 (13%) had juvenile myoclonic epilepsy (e.g., Fig. 2D). Typical juvenile myoclonic epilepsy and absence epilepsies occurred together in 22 (19%) families, including six families containing individuals (N=7) whose epilepsy evolved from absence epilepsy (CAE or CAE/Juvenile absence epilepsy indistinguishable) to JME. Three families had generalized tonic-clonic seizures alone. The remaining families were more mixed: 13 families had more severe forms of genetic generalized epilepsy in at least one family member (e.g., Fig. 2E), and in 16 families a specific familial electroclinical epilepsy syndrome could not be determined.
PHENOTYPES IN 303 EPILEPSY FAMILIES

Formal testing of the hypothesis that absence epilepsies and juvenile myoclonic epilepsy segregated separately into different families provided significant evidence for familial clustering of each syndrome (Krippendorff’s alpha=0.67; resampling-based \( p<0.0001 \)). This finding remained significant (Krippendorff’s alpha=0.65; resampling-based \( p<0.0001 \)) when 16 generalized families included in earlier small studies analyzing segregation of seizure types or syndromes were excluded from the analysis (Winawer et al., 2003b, Marini et al., 2004, Winawer et al., 2005).

Within the 48 families with absence epilepsy, individual families generally showed a mixture of absence phenotypes, including early onset absence epilepsy, childhood absence epilepsy, childhood/juvenile absence epilepsy and juvenile absence epilepsy. Whilst some families segregated a single absence syndrome, there was no strong evidence of assorting separately into families overall.

Thirteen families had family members with severe phenotypes; 8/14 (57%) of individuals with severe genetic generalized epilepsy phenotypes (epilepsy with eyelid myoclonias, MAE, or myoclonic absence epilepsy) had mild intellectual disability or developmental delay, as did 6/25 (24%) of their relatives with GGE. This frequency of developmental delay in these 25 relatives was greater than the frequency of intellectual impairment in individuals with GGE in the remaining generalized families 23/287 (8%) but the difference was not significant after multiple testing (\( p=0.63 \), GEE-based Wald test=4.15, \( df=1 \)). Data on intellect was not available for one individual with eyelid myoclonia and absences and 11 genetic generalized epilepsy cases. It should be noted that intellectual functioning was not systematically assessed.

Finally, because photosensitivity has been proposed as an endophenotype (von Spiczak et al., 2011) we noted that 14/117 families contained two or more individuals who reported seizures triggered by light stimulation. The syndromes in photosensitive individuals included absence epilepsies, epilepsy with eyelid myoclonias, juvenile myoclonic epilepsy and generalized tonic-clonic seizures alone.

Syndromes in familial focal epilepsy

Families were categorized according to a specific localization only when all affected family members had the same localization (although some individuals could have unclassifiable epilepsy or unlocalized focal epilepsy) (e.g., Fig. 2F). Among the 62 families with focal epilepsy, 37% (\( n=23 \)) had familial temporal lobe epilepsy (Table 4). In 18 of the 23 families with familial temporal lobe epilepsy, most individuals had mesial temporal features (e.g. déjà vu, epigastric sensation, olfactory aura). MRI data were available on 40/66 cases within these 18 families; all images were reported as normal, although volumetric studies were not done and images were not available for systematic
PHENOTYPES IN 303 EPILEPSY FAMILIES

review. Two families contained two or more members with lateral temporal features (e.g., auditory aura, aphasic seizures). Three families had a mixture of individuals with features suggestive of lateral and mesial temporal lobe epilepsy.

The remaining families had a variety of localizations (Table 4). Three (5%) families had frontal lobe epilepsy. Four families had familial occipital epilepsy, three had occipito-temporal lobe epilepsy, and seven had temporo-parietal features (e.g., Fig. 2G). We aggregated these 15 families as “posterior quadrant epilepsies” due to anatomical overlap. One case of IPOE without generalized epilepsy features was observed in the focal epilepsy families, in a familial occipital epilepsy family.

Five families (8%) had multiple affected members with self-limited focal epilepsy of childhood (Fig. 2H). Interestingly, all but one of these families had horizontal pedigrees consistent with recessive inheritance. Two families had multiple members with typical childhood epilepsy with centrotemporal spikes, two had atypical childhood epilepsy with centrotemporal spikes, and one had individuals with childhood epilepsy with centrotemporal spikes or occipital foci.

Seven families (11%) had heterogeneous localizations. Of the 19 classified individuals in these families, five (four families) had typical or atypical childhood epilepsy with centrotemporal spikes, four individuals (four families) had posterior quadrant epilepsy (three occipital, one parietal), three individuals had frontal lobe epilepsy and only three individuals were diagnosed with temporal lobe epilepsy. The remaining nine families had insufficient clinical detail to localize the focal epilepsy in more than one family member.

GEFS+ families

Twenty-two families were classified as GEFS+ (see Methods for specific definition). Analysis of epilepsy phenotypes and segregation patterns within the pedigrees suggested that these families could be divided into two subgroups. The first subgroup (n=14) comprised classical GEFS+ spectrum families where individuals had FS+, FS alone, or epilepsy phenotypes (e.g. absence epilepsies, temporal lobe epilepsy) that have been reported within the GEFS+ spectrum, and pedigree analysis was consistent with simple autosomal dominant inheritance (e.g., Fig. 2I). The second subgroup (n=8) comprised GEFS+ families with unusual features such as bilineal inheritance (n=5; Fig. 2J) or phenotypes not generally recognized within the GEFS+ spectrum (e.g., occipital epilepsy) (e.g., Fig. 2K).

These 22 families contained 96 individuals with epilepsy: 24 individuals with FS+, 26 with febrile seizures with subsequent epilepsy (generalized, focal or unclassified), and 46 with epilepsies without known early febrile seizures, although early histories were often incomplete. Finally, an
PHENOTYPES IN 303 EPILEPSY FAMILIES

additional 22 individuals had FS alone. Generalized epilepsy phenotypes in the GEFS+ cohort were predominantly absence syndromes, including early onset absence epilepsy (Table 1). Although this ‘new’ phenotype has not been explicitly diagnosed in GEFS+ pedigrees, it has been previously observed in families, including some with pathogenic GABA receptor variants (Marini et al., 2003). Epilepsy with myoclonic-ataonic seizures was seen in four individuals, again a phenotype well-documented in GEFS+ families with ion channel mutations (Wallace et al., 1998, Scheffer et al., 2001).

Pedigree structures

Pedigree configurations of the 303 families according to the four broad familial epilepsy categories are shown in Table 5. 47/303 (16%) had a horizontal structure (e.g. Fig. 2D), where only siblings or siblings and first cousins were affected. Nine of these 47 families were consanguineous, supporting a recessive mode of inheritance. 28/303 families (9%) had three sequential generations affected with epilepsy, consistent with a dominant mode of inheritance (e.g. Fig. 2E). 24/303 families (8%) had a bilineal family history of seizures.

The number of meiotic events between the most distantly-related affected individuals did not differ among familial epilepsy categories when all four categories were included in the analysis ($p=0.13$, Kruskal- Wallis $\chi^2=11.70$, $df=0.3$). However, in comparisons of mixed families with the other three familial epilepsy categories combined, the number of meiotic events between the most distantly-related affected individuals was greater in the mixed families ($p=0.022$, Kruskal-Wallis $\chi^2=10.13$, $df=1$). Individuals of third-degree or higher relationship were present in 46/102 (45%) of mixed families and 50/201 (25%) of non-mixed families ($p=0.0078$, Wald test=12.04, $df=1$). In addition, affected family members in generalized families appeared more closely related than the other three familial epilepsy categories, with 90/117 (77%) of generalized families containing only first- or second-degree relatives compared to 117/186 (63%) of families in the other three categories, although this trend was not significant after adjustment for multiple testing ($p=0.084$, Wald test=7.68, $df=1$).

Sex ratios and onset age in the four broad familial epilepsy categories

Within these families, we observed significantly more females (n=637) than males (n=483) with epilepsy ($p=0.00014$, GEE-based Wald test=19.52, $df=1$). Further investigation revealed this result was driven primarily by an excess of affected females in GGE families (259 females, 158 males, $p<0.0001$, GEE-based Wald test=22.36, $df=1$). This excess of affected females in GGE families
PHENOTYPES IN 303 EPILEPSY FAMILIES

remained significant when 26 generalized families included an earlier study where a sex bias was observed (Afawi et al., 2016) were excluded from the analysis (199 females, 120 males, $p<0.0001$, GEE-based Wald test=20.55, $df=1$). Mixed families (218 females, 169 males, $p=0.15$, GEE-based Wald test=6.68, $df=1$) also showed a trend of female excess although the result was not significant after adjusting for multiple testing. We observed no evidence of sex difference in focal families ($p=1.00$, GEE-based Wald test=0.69, $df=1$) or GEFS+ families ($p=1.00$, GEE-based Wald test=0.14, $df=1$).

Onset ages of seizures in individuals within broad familial classifications are shown in Table 2 and Figure 3. We observed significant differences in the mean age of onset among the familial epilepsy categories ($p=0.0014$, GEE-based Wald test=21.18, $df=3$) with mean age at onset significantly younger in GEFS+ families (7.77 years) than others (12.12 years) ($p=0.009$, GEE-based Wald test=11.85, $df=1$) and mean age at onset significantly older in focal epilepsy families (mean of 15.3 years) than others (mean of 10.9 years) ($p=0.0073$, GEE-based Wald test=12.17, $df=1$). Onset in GGE and focal epilepsy families peaked during early adolescence (10-14 years). Looking across the lifespan, of the 117 GGE families, onset of seizures was skewed towards early childhood (under 10 years), reflecting an enrichment of early onset and childhood absence epilepsies in this cohort. In contrast, families with focal epilepsy showed a more normal distribution, with onset of seizures more distributed across early childhood and adulthood. The majority of individuals within GEFS+ families had onset of unprovoked seizures under 10 years.
PHENOTYPES IN 303 EPILEPSY FAMILIES

Discussion

This multi-center study of 303 multiplex families with non-acquired epilepsies illustrates the complex phenotypic relationships among common epilepsies, essential for understanding the genetic architecture of these disorders. Although the number of contributing families from the sites differed, the patterns observed were similar across the different sites.

Broad classification of families

Over one third of these multiplex families had GGE in all classified individuals (Table 1); this is consistent with earlier knowledge from familial aggregation (Peljto et al., 2014) and twin studies (Berkovic et al., 1998, Kjeldsen et al., 2003, Vadlamudi et al., 2004a, Vadlamudi et al., 2014) showing that GGE has the highest heritability of the common epilepsies. One fifth of the cohort were families with ‘pure’ focal epilepsies, generally with homogeneous syndromes within families (Table 5). This reinforces the importance of genetic factors in certain focal epilepsies, in contrast to the outdated view that these are largely acquired disorders.

Whilst separate segregation of generalized and focal epilepsy was mainly observed across the cohort, one third of families (99 families) had evidence of both generalized and focal features, and three families had FS+ and other syndromes where a diagnosis of GEFS+ could not be made. Chance occurrence of unrelated epilepsies likely accounts for some of our mixed families. This is most probable in some of the 21 families where more than two meioses separated individuals with different major syndromes (Fig. 1) and the observation that mixed families overall showed more meiotic events between the most distantly related affected individuals than non-mixed families.

Evidence from genetic epidemiology studies and smaller family studies has suggested both shared and distinct genetic susceptibility to generalized and focal epilepsies (Ottman et al., 1998, Winawer et al., 2003a, Peljto et al., 2014). Both focal and generalized epilepsies have been observed in large families segregating pathogenic variants of major effect (e.g., GABRG2 (Marini et al., 2003), SLC2A1 (Mullen et al., 2010), SCN1B (Scheffer et al., 2007)), and occurrence of mixed epilepsies in single individuals has been reported (Koutroumanidis et al., 1999, Nicolson et al., 2004, Radhakrishnan et al., 2011). Among the 99 families with both generalized and focal epilepsy features in our study, 78 (79%) contained mixed epilepsy types within individuals or closely-related family members, which is less likely to be due to chance and suggestive of shared genetic susceptibility to both types of epilepsy.

The coexistence of focal and generalized epilepsy within individuals is considered rare, with frequencies in case series of around 1% (Koutroumanidis et al., 1999, Nicolson et al., 2004, Radhakrishnan et al., 2011). Among the 1120 individuals in this study, 39 (3.5%) of individuals in
PHENOTYPES IN 303 EPILEPSY FAMILIES

102 families had features of both focal and generalized epilepsy. IPOE, observed in 20 individuals in 13 of these mixed families, presents an interesting challenge to classification and understanding the neurobiological separation of generalized and focal epilepsies. Individuals have focal (occipital) seizures, photosensitivity which is usually a generalized trait, and often generalized seizures (i.e. generalized tonic-clonic and/or myoclonic seizures) and generalized spike and wave discharges. Earlier studies suggest that IPOE is biologically related to generalized epilepsy (see Methods); indeed in 9/13 (69%) families IPOE exclusively co-segregated with juvenile myoclonic epilepsy and other generalized syndromes, however cases with exclusively focal epilepsy (temporal and frontal lobe epilepsy) in their family members were also seen. Similarly, self-limited focal epilepsies of childhood, whilst classically considered focal epilepsies, can have features overlapping with generalized syndromes (Bray and Wiser, 1965, Degen and Degen, 1990, Yum et al., 2010, Cerminara et al., 2012). Co-occurrence with generalized epilepsies in 13 ‘mixed’ families here is reflective of this, and may suggest shared genetic determinants in the context of the ill-understood genetic architecture of the self-limited focal epilepsies of childhood (see above).

The boundaries of the familial syndrome of GEFS+ are indistinct and controversial (Thomas et al., 2012). Moreover, although inheritance is autosomal dominant in the majority of reported cases, inheritance with known mutations of large effect size in SCN1A, SCN1B, GABARG2 or STX1B is well known, and family and twin studies also indicate complex inheritance in some families (Singh et al., 1999, Eckhaus et al., 2013). Some large GEFS+ families with known pathogenic mutations in ion channel genes contain individuals with generalized or focal epilepsies in addition to the core phenotypes of FS+ and FS, showing that these epilepsies can also be part of the GEFS+ spectrum (Wallace et al., 1998, Baulac et al., 1999, Scheffer et al., 2001, Scheffer et al., 2007). Here we observed 14 ‘classical’ GEFS+ families with 3-7 family members with FS, FS+ or other epilepsies recognized within the GEFS+ spectrum, segregating in a manner compatible with dominant inheritance; none had known variants in the established genes, although the screening was not systematic nor with contemporary techniques. Other families were difficult to classify: five families had evidence of bilineal inheritance, which has been previously noted in GEFS+, and three families had additional phenotypes, such as childhood epilepsy with centrottemporal spikes, occipital epilepsy, juvenile absence epilepsy and juvenile myoclonic epilepsy, raising the question as to whether these phenotypes can also sometimes be part of the GEFS+ spectrum or whether such observations are due to chance association. Gene discovery in these families will be especially helpful to clarify the boundaries and phenotype-genotype correlations of the GEFS+ spectrum.
PHENOTYPES IN 303 EPILEPSY FAMILIES

Segregation of syndromes in generalized epilepsy families

Absence epilepsies were shown to aggregate preferentially with each other and not with JME. Similarly, individuals with JME were more likely to have relatives with JME than absence phenotypes. This confirms earlier observations in smaller cohorts (Marini et al., 2004, Winawer et al., 2005). In addition to the observation of ‘like-with-like’ in generalized epilepsy families, these families tended to be more closely related than the other families although this observation did not reach statistical significance.

Early onset absence epilepsy, defined as onset under age four years, was observed in 10% of affected individuals in generalized epilepsy families as well as many GEFS+ and mixed families (Table 1). Within families, early onset absence epilepsy segregated with itself or other absence syndromes. This disorder is not specifically recognized in the current ILAE list of syndromes or diagnostic manual (Berg et al., 2010, ILAE Diagnostic Manual, https://www.epilepsydiagnosis.org), but is at least partly incorporated as the early extreme of childhood absence epilepsy. Its importance has been brought into focus by discovery of glucose transporter defects in 10% of cases (Suls et al., 2009, Arsov et al., 2012), suggesting some biological separation from classical CAE. The frequency of early-onset absence epilepsy was higher than we anticipated; most were pre-screened for glucose transporter defects and those with pathogenic variants were excluded from the study.

The ‘severe’ syndrome of MAE aggregated with absence epilepsies. This syndrome is also known to occur in families with GEFS+, including dominant families with known sodium channel mutations (Wallace et al., 1998, Scheffer et al., 2001). These data suggest that MAE may share genetic determinants with classical GGE as well as with GEFS+, especially as only 1/4 of the cases in GGE families had a family history of febrile seizures. Similarly, the syndrome of epilepsy with eyelid myoclonias has also been described as occurring particularly within GEFS+ families, in addition to some aggregation with classical GGE phenotypes (Sadleir et al., 2012). Here, we observed this disorder mainly with GGE; the difference in emphasis may be an artifact of ascertainment and methodology.

Familial focal epilepsy syndromes

Of the 62 familial focal epilepsy families, 46 could be categorized into familial regional syndromes (Table 5). These were defined as having two or more individuals with the same localization and no others with a different localization. Familial TLE was the most common form (n=23), perhaps not a surprising finding given that temporal lobe epilepsy is the most common focal epilepsy. These families largely comprised those with mesial temporal semiology; there were only two families with features strongly suggestive of lateral TLE, where pathogenic variants in LGII might be suspected. The separation of mesial and lateral TLE was not absolute as three families had features of both. We
PHENOTYPES IN 303 EPILEPSY FAMILIES

were struck by the observation of 15 families with semiologies arising from the parietal occipital or occipito-temporal regions, and grouped them as familial posterior quadrant epilepsies. Although rare individual families segregating occipital epilepsy (Kuzniecky and Rosenblatt, 1987, Deprez et al., 2007, Grosso et al., 2008) or ‘peri-central foci’ (Kinton et al., 2002) have been reported, our findings suggest such posterior quadrant epilepsies may be more common and potential targets for novel gene discovery.

It is known that families can have different regional focal epilepsies in family members, most notably in the syndrome of autosomal dominant focal epilepsy with variable foci due to DEPDC5 mutations (Dibbens et al., 2013). We also observed seven families with different regional epilepsies; some have already had DEPDC5 mutations excluded suggesting there are other genes to be discovered.

Self-limited focal epilepsies of childhood, particularly childhood epilepsy with centrotemporal spikes, remain a mystery regarding their etiology. Traditionally classified as ‘idiopathic’, suggesting a genetic etiology, case series rarely show a family history of the same disorder and twin studies show very low concordance in identical twins (Vadlamudi et al., 2006, Vears et al., 2012). Most cases appear sporadic or have a family history of febrile seizures or a relationship to generalized epilepsies (Ma and Chan, 2003, Vadlamudi et al., 2006, Vears et al., 2012). Some familial cases are known and GRIN2A pathogenic variants have been found in a small minority of cases, although these cases are often more severe and are related to the epilepsy-aphasia spectrum (Carvill et al., 2013, Kingwell, 2013, Lesca et al., 2013). We observed five families, four with a ‘horizontal’ pedigree suggesting possible recessive inheritance (e.g., Fig. 3C); none had the more complex recessive phenotype described with exercise-induced dystonia (Guerrini et al., 1999). Some variants have been reported that may raise the risk for these epilepsies (Panjwani et al., 2016); our collection of families suggests an opportunity to find genes of large effect size that may shed light on the biology of these enigmatic common epilepsies.

Onset ages and sex ratios

Age of onset of epilepsy in family members was predominantly in the first two decades of life (Table 2). Three quarters of individuals in GEFS+ families had epilepsy onset (excluding febrile seizures) under 10 years. Individuals from GGE families had onsets evenly spread across the first two decades, with a few late onset cases. Individuals from focal epilepsy families had a larger proportion of cases with onset over age 20 years compared to GGE and GEFS+ families (Fig. 3). The different spectrum of onset ages might be due to differences in age-specific expression of the genes involved; emerging knowledge of the developmental expression of genes (Thompson et al., 2014) could be used to rank putative candidate variants.
PHENOTYPES IN 303 EPILEPSY FAMILIES

The sex ratio of affected individuals was skewed towards females. This was driven by the GGE families (Table 2). A predominance of females in GGE has been reported (Christensen et al., 2005, McHugh and Delanty, 2008, Afawi et al., 2016). Sex ratios may be subject to ascertainment bias, especially for studies of singletons as males may be more difficult to recruit. Bias may be less likely in family studies; indeed that the female predominance in GGE families is not due to ascertainment bias is supported by the equal sex ratios in GEFS+ and focal families, consistent with previous reports of no sex difference in localization-related epilepsy in both population and twin data (Christensen et al., 2005). These findings indicate that there is a biologically sex-related difference in GGE which needs to be accounted for in genetic models of generalized epilepsy.

Limitations

Ascertainment was intentionally targeted to identify ‘genetically enriched’ pedigrees with three or more affected individuals, so this cohort is not representative of all individuals with epilepsy. Identification of affected family members was limited by the knowledge of affected status by other family members; mildly affected members or those that chose not to disclose their seizures may have been missed. Collection and interpretation of phenotypic data was restricted by access to comprehensive medical records, availability of family members for interview and their accurate recollection and description of events. Where these phenotypic limitations of reliable information were identified, classification of the epilepsy syndrome remained conservative.

Future direction

This large phenotypic data set provides an important resource for molecular genetic analyses of these disorders. New familial focal epilepsy syndromes have been identified, and segregation of phenotypes and sex differences within the genetic generalized epilepsies provide clues for deciphering the genetic determinants of these common epilepsies. The overlap of the generalized and focal epilepsy syndromes within individuals and families suggests that some genes may influence risk for both generalized and focal epilepsy. The structure of some of these families, with suggestive dominant or recessive segregation, indicates that putative novel genes of large effect are yet to be discovered for common epilepsies in some families. Yet, data from familial aggregation and twin studies show that Mendelian inheritance is unlikely for the majority of cases. Solving the genetic architecture of common complex diseases remains a major challenge in the genetics field broadly (Zuk et al., 2014, Fuchsberger et al., 2016). The findings here suggest that analysis of phenotypic patterns within and across these multiplex families may be strategic in reducing the genomic search space for forthcoming analyses of next generation sequence data.
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PHENOTYPES IN 303 EPILEPSY FAMILIES

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PHENOTYPES IN 303 EPILEPSY FAMILIES

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PHENOTYPES IN 303 EPILEPSY FAMILIES

References


PHENOTYPES IN 303 EPILEPSY FAMILIES


PHENOTYPES IN 303 EPILEPSY FAMILIES


PHENOTYPES IN 303 EPILEPSY FAMILIES


PHENOTYPES IN 303 EPILEPSY FAMILIES


PHENOTYPES IN 303 EPILEPSY FAMILIES


Figure legends

Figure 1. ‘Mixed’ Families. The minimum number of meiotic events between family members with focal and generalized epilepsy phenotypes are shown for 99 families.

Figure 2. Examples of families with mixed epilepsy syndromes (A=generalized and focal epilepsy in same individual, B=sibship of generalized epilepsy and self-limited focal epilepsy of childhood, C=focal and generalized epilepsy syndromes segregating in distantly related family members (>3 meiosis), familial generalized epilepsy (D,E), familial focal epilepsy (F=familial temporal lobe epilepsy, G=familial occipito-temporal epilepsy, H=familial self-limited focal epilepsy of childhood), and GEFS+ (I=typical GEFS+, J=bilineal family history of FS, K=GEFS+ family with atypical phenotypes). CAE=childhood absence epilepsy; JAE=juvenile absence epilepsy; CAE/JAE =CAE/JAE indistinguishable; CECTS=childhood epilepsy with centrotemporal spikes; EMA=epilepsy with eyelid myoclonias; EOAE=early onset absence epilepsy; FS+=febrile seizures plus; JME=juvenile myoclonic epilepsy; MAE=epilepsy with myoclonic-atonic seizures; OLE=occipital lobe epilepsy; TLE=temporal lobe epilepsy.

Figure 3. Age of onset of individuals affected with unprovoked seizures within familial epilepsy groupings. Data includes ages of onset of unclassified individuals within each syndromic group.