Supplementary Data

Common risk variants for epilepsy are enriched in families previously targeted for rare monogenic variant discovery

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Supplementary Table 1. Summary of original samples by cohort origin and SNP array.

Sample source	Illumina array	Number samples	Case / Control
Epi4K families	HC, HME, MEGA	1,784 (303 families)	Cases + unaffected relatives
University of Melbourne families	GSA	223 (36 families)	Cases + unaffected relatives
Epi4K singletons	HC	488	Cases
Tertiary hospital singletons (Australia, NZ, USA)	GSA	2,132	Cases
QSkin	GSA	17,233	Controls

Abbreviations: GSA, Global Screening Array; HC, HumanCore; HME, Human MultiEthnic; MEGA, MultiEthnic Global Array.

Supplementary Table 2. Demographics of final study cohorts under comparison.

Group	N	Sex, M:F	Age[a], range (median)	Country of origin[b]
Familial Epilepsy Cases	1,818	45%:55%	0–101 years (31)	Australia, Ireland, Israel, New Zealand, USA, Wales
Sporadic Epilepsy Cases	1,182	50%:50%	0–82 years (29)	Australian, New Zealand, USA
Unaffected Relatives	771	44%:56%	18–97 years (55)	Australia, Ireland, Israel, New Zealand, USA, Wales
Controls	15,929	45%:55%	40–69 years (NA)	Australia

[a]Age in years at study recruitment. Extreme age ranges reflect extensive recruitment of familial relatives (affected and unaffected).
[b]By this stage, samples had been restricted to those of European genetic ancestry (see Methods).

Supplementary Table 3. Detailed phenotypes of epilepsy cases.

Epilepsy Phenotype	N	Syndromes included
Genetic Generalised Epilepsies (GGE) (n = 922)		
Absence epilepsies	206	CAE, JAE
Juvenile myoclonic epilepsy	100	JME
Other GGE	78	GTCSA, late onset GGE
Unspecified GGE	538	
Focal Epilepsies (n = 1,276)		
Lesional focal epilepsies		
- MCD	201	FCD, PMG, PVNH
- Benign neoplasm	47	DNET, ganglioglioma
- Other lesional epilepsies	101	TBI, stroke, unspecified lesional
Non-lesional focal epilepsies		
- SLFEC	39	BECTS, childhood occipital epilepsies
- Temporal lobe epilepsy	237	
- Other localised	60	Frontal, parietal, occipital epilepsies
Unspecified focal epilepsies	591	
Other Epilepsies (n = 802)		
DEE		
- Infantile spasms	162	
- Lennox-Gastaut syndrome	138	
- Other DEE	174	Dravet, MAE, CSWS
Febrile seizures-plus	57	
Febrile seizures alone	127	
Combined generalised/focal epilepsy	35	
Unclassified epilepsy	109	

Abbreviations:
BECTS, benign epilepsy of childhood with centro-temporal spikes
CAE, childhood absence epilepsy
CSWS, continuous spike-and-wave during sleep
DEE, developmental and epileptic encephalopathies
DNET, dysembryoplastic neuroectodermal tumor
FCD, focal cortical dysplasia
GGE, genetic generalised epilepsy
GTCSA, generalised tonic-clonic seizures alone
JAE, juvenile absence epilepsy
JME, juvenile myoclonic epilepsy
MAE, myoclonic-atonic epilepsy
MCD, malformations of cortical development
MFSI, migrating focal seizures of infancy
PMG, polymicrogyria
PVNH, periventricular nodular heterotopia
SLFEC, self-limited focal epilepsies of childhood
TBI, traumatic brain injury

Supplementary Table 4. Deciles of polygenic risk.
Decile cut-points were determined from the PRS distribution of population controls, then applied to other study cohorts with the lowest decile of the particular study cohort used as a reference. Odds ratios were derived from mixed-effects logistic regression models, accounting for family relatedness, sex and principal components of ancestry as covariates.

a. Familial epilepsy cases

Decile	N cases	N controls	OR	95% CI
1	127	1,593	Ref.	n/a
2	155	1,593	1.22	0.95, 1.58
3	144	1,593	1.09	0.85, 1.42
4	150	1,593	1.13	0.88, 1.46
5	142	1,593	1.08	0.84, 1.40
6	182	1,593	1.44	1.13, 1.85
7	182	1,593	1.37	1.08, 1.76
8	199	1,593	1.44	1.13, 1.84
9	218	1,593	1.55	1.22, 1.97
10	319	1,593	2.06	1.65, 2.60
Top 1%	*63*	*160*	*3.80*	*1.92, 5.69*

b. Sporadic epilepsy cases

Decile	N cases	N controls	OR	95% CI
1	95	1,593	Ref.	n/a
2	112	1,593	1.14	0.85, 1.53
3	98	1,593	0.97	0.71, 1.31
4	98	1,593	0.97	0.72, 1.32
5	105	1,593	0.99	0.74, 1.34
6	137	1,593	1.35	1.02, 1.80
7	126	1,593	1.21	0.91, 1.62
8	125	1,593	1.10	0.82, 1.48
9	117	1,593	1.02	0.76, 1.37
10	169	1,593	1.41	1.07, 1.86
Top 1%	*23*	*160*	*1.80*	*1.03, 3.12*

c. Unaffected relatives of familial epilepsy cases

Decile	N relatives	N controls	OR	95% CI
1	58	1,593	Ref.	n/a
2	74	1,593	1.24	0.87, 1.78
3	80	1,593	1.38	0.96, 1.97
4	80	1,593	1.33	0.94, 1.91
5	78	1,593	1.31	0.93, 1.87
6	72	1,593	1.21	0.85, 1.74
7	65	1,593	1.14	0.79, 1.63
8	69	1,593	1.07	0.75, 1.55
9	84	1,593	1.30	0.92, 1.85
10	111	1,593	1.62	1.16, 2.27
Top 1%	*20*	*160*	*1.95*	*0.98, 3.91*

Supplementary Table 5. Varying model thresholds.
We repeated the primary analysis, comparing familial cases (n = 1,818) to population controls (n = 15,929) using a range of thresholds to determine which variants to include in the polygenic risk score model. P-values were adjusted for multiple comparisons (FDR < 0.05).

Threshold	SNPs included	OR (95% CI)	p_{adj}
<0.5	59,138	1.20 (1.13, 1.27)	5×10^{-9}
<0.1	23,722	1.20 (1.13, 1.27)	4×10^{-9}
<0.05	14,837	1.19 (1.12, 1.26)	1×10^{-8}
<0.01	5,189	1.17 (1.10, 1.24)	4×10^{-7}
$<1 \times 10^{-5}$	44	1.07 (1.02, 1.13)	0.03
$<1 \times 10^{-7}$	6	1.08 (1.02, 1.14)	0.02

Supplementary Table 6. Negative control, Asthma PRS.

Group	Asthma PRS, Mean (SD)	OR	95% CI	p-value
Controls	0.00 (1.00)	Ref.	n/a	n/a
Familial Epilepsy Cases	0.03 (0.99)	0.96	0.91, 1.01	0.14
Sporadic Epilepsy Cases	0.01 (1.00)	1.04	0.97, 1.10	0.23
Unaffected Relatives	0.02 (1.05)	0.97	0.89, 1.06	0.54

OR = Odds ratio of being in the specified case group, compared to being in the control group, for every one unit (standard deviation) increase in Asthma PRS, after adjusting for ancestry, sex, and family relatedness.

Supplementary Table 7. PRS Models stratified by epilepsy phenotype.

a. Epilepsy-PRS Model

Group	N	PRS mean (95% CI)	OR (95% CI)[a]	p_{adj}
Controls	15,929	0	Ref	n/a
GGE, Familial	678	0.42 (0.34, 0.50)	1.33 (1.22, 1.44)	7×10^{-10}
GGE, Sporadic	244	0.33 (0.20, 0.46)	1.27 (1.12, 1.44)	9×10^{-4}
Focal, Familial	601	0.17 (0.08, 0.26)	1.11 (1.02, 1.21)	0.03
Focal, Sporadic	675	0.10 (0.02, 0.18)	1.02 (0.95, 1.11)	0.63
Other, Familial	539	0.23 (0.14, 0.31)	1.15 (1.05, 1.26)	0.005
Other, Sporadic	263	0.14 (0.07, 0.22)	1.07 (0.95, 1.21)	0.36

b. GGE-PRS Model

Group	N	PRS mean (95% CI)	OR (95% CI)[a]	p_{adj}
Controls	15,929	0	Ref	n/a
GGE, Familial	678	0.60 (0.51, 0.70)	1.58 (1.45, 1.72)	6×10^{-25}
GGE, Sporadic	244	0.47 (0.34, 0.60)	1.46 (1.28, 1.65)	5×10^{-8}
Focal, Familial	601	0.11 (0.02, 0.19)	1.05 (0.96, 1.14)	0.36
Focal, Sporadic	675	0.13 (0.05, 0.20)	1.04 (0.96, 1.12)	0.42
Other, Familial	539	0.27 (0.18, 0.36)	1.25 (1.14, 1.37)	4×10^{-6}
Other, Sporadic	263	0.22 (0.11, 0.33)	1.12 (0.99, 1.27)	0.13

c. FE-PRS Model

Group	N	PRS mean (95% CI)	OR (95% CI)[a]	p_{adj}
Controls	15,929	0.00 (-0.02, 0.02)	Ref.	n/a
GGE, Familial	678	0.17 (0.09, 0.25)	1.05 (0.96, 1.14)	0.37
GGE, Sporadic	244	0.12 (-0.01, 0.26)	1.04 (0.91, 1.18)	0.66
Focal, Familial	601	0.15 (0.07, 0.23)	1.09 (1.00, 1.18)	0.11
Focal, Sporadic	675	0.09 (0.01, 0.17)	1.02 (0.94, 1.10)	0.76
Other, Familial	539	0.12 (0.03, 0.20)	1.04 (0.95, 1.14)	0.42
Other, Sporadic	263	0.05 (-0.08, 0.17)	0.99 (0.88, 1.12)	0.91

[a]Odds ratios are for the comparison to population controls, adjusted for sex, five ancestry principal components, and genetic relatedness within families. P-values were adjusted for multiple comparisons (FDR < 0.05).

Supplementary Table 8. Epilepsy types among rare variant carriers, familial cases.

Epilepsy Type	RV positive	RV negative
GGE	20	658
Focal	31	570
Other		
- DEE	51	169
- Febrile seizures-plus	32	147
- Combined	0	32
- Unclassified	6	102
Total	140	1,678

Abbreviations: GGE, genetic generalised epilepsies; DEE, developmental and epileptic encephalopathy; RV, rare variant (pathogenic single nucleotide variant or recurrent epilepsy-associated microdeletion).

Supplementary Table 9. PRS in cases carrying rare epilepsy variants.

a. Familial cases

Polygenic risk scores in individuals with familial epilepsy who were positive for rare variants (n = 140) versus individuals negative for rare variants (n = 1,678). P-values adjusted for multiple comparisons (FDR < 0.05) were not significant.

PRS Model	OR	95% CI	p_{adj}
All-Epilepsy-PRS	0.90	0.74, 1.10	0.37
GGE-PRS	0.91	0.74, 1.11	0.40
FE-PRS	0.86	0.71, 1.03	0.21

OR = odds ratio of being in the rare variant-positive group, compared to being in the rare variant-negative group, for every one unit (standard deviation) increase in PRS, after adjusting for ancestry, sex, and family relatedness.

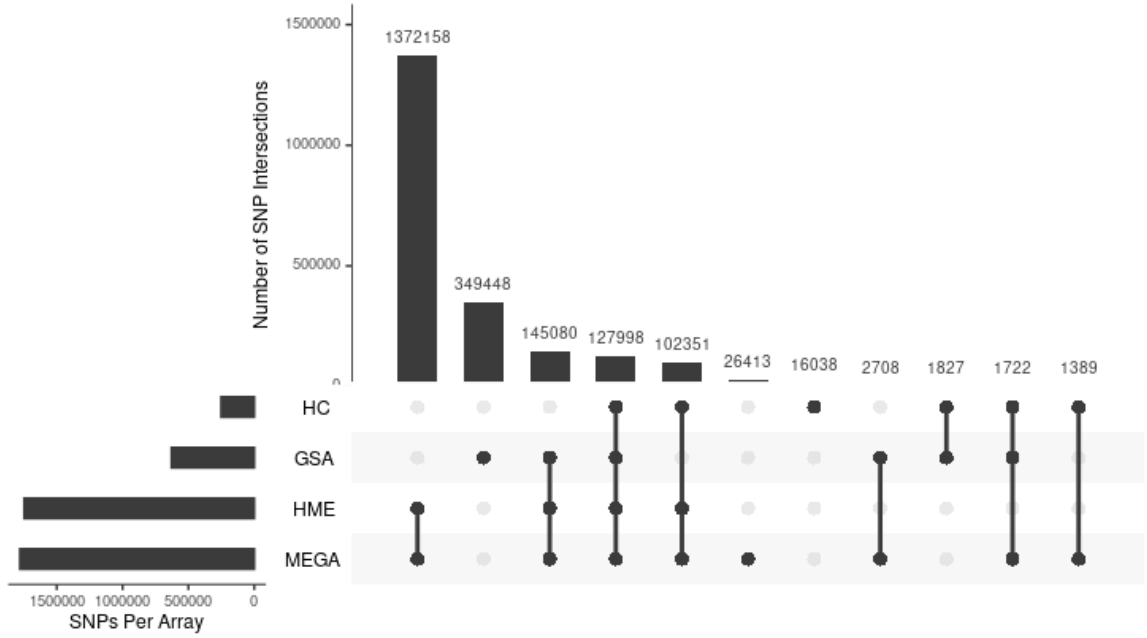
b. Sporadic cases

Polygenic risk scores in individuals with sporadic epilepsy who were positive for rare variants (n = 48) versus individuals negative for rare variants (n = 1,134). P-values adjusted for multiple comparisons (FDR < 0.05) were not significant.

PRS Model	OR	95% CI	p_{adj}
All-Epilepsy-PRS	0.98	0.73, 1.31	0.91
GGE-PRS	0.77	0.58, 1.07	0.13
FE-PRS	1.00	0.74, 1.35	0.98

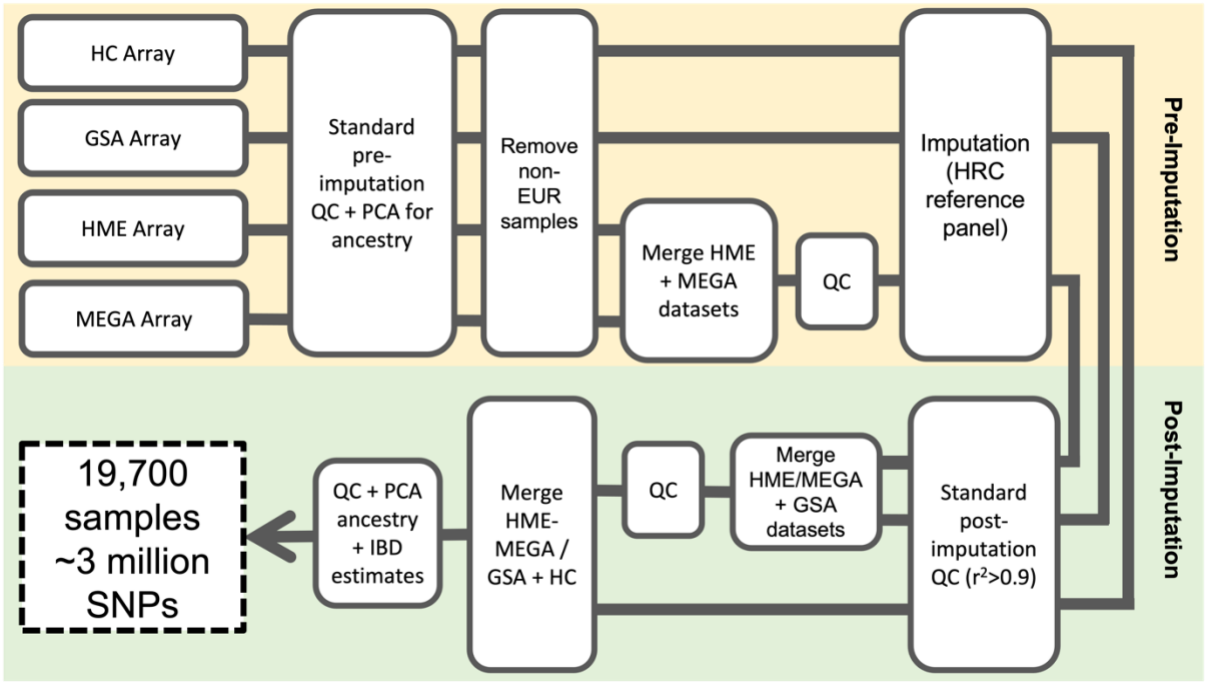
OR = odds ratio of being in the rare variant-positive group, compared to being in the rare variant-negative group, for every one unit (standard deviation) increase in PRS, after adjusting for ancestry and sex.

Supplementary Figure 1. SNP concordance across four Illumina array types.
The four arrays genotyped 127,998 SNPs in common, with greatest concordance (98.2%) between the two arrays with the most SNPs, HME and MEGA.



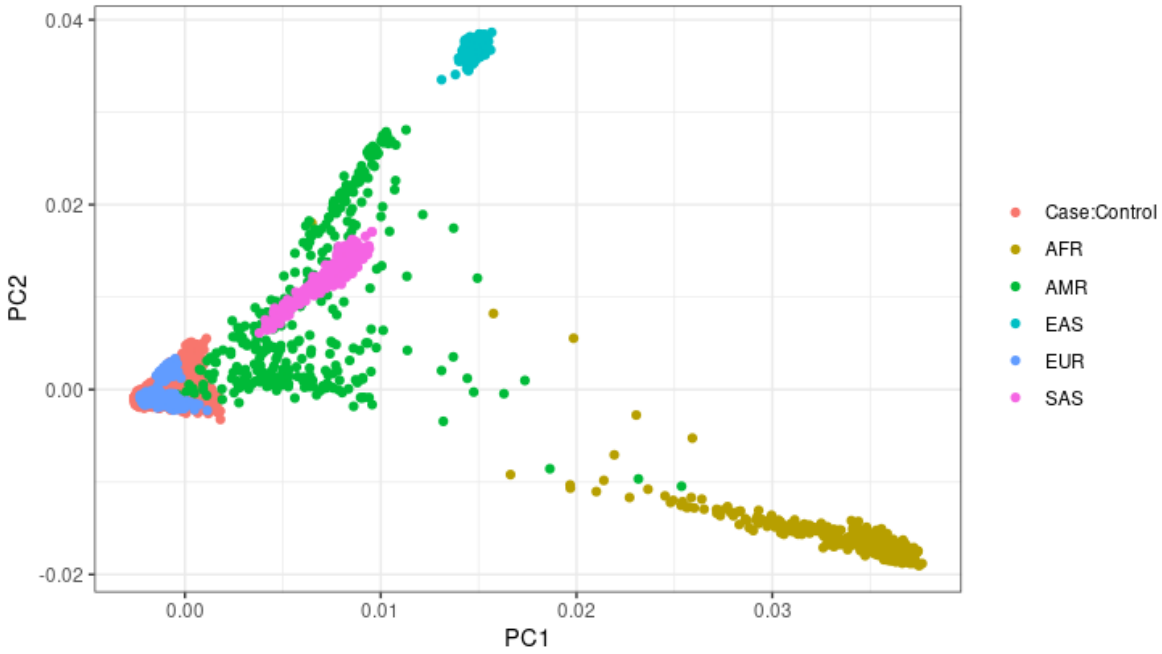
HC: HumanCore (n=251,325 SNPs); GSA: Global Screening Array (n=628,783 SNPs); HME: Human Multi-Ethnic (n=1,747,587 SNPs); MEGA: Multi- Ethnic Global Array (n=1,779,819 SNPs).

Supplementary Figure 2. Quality control and SNP imputation workflow.



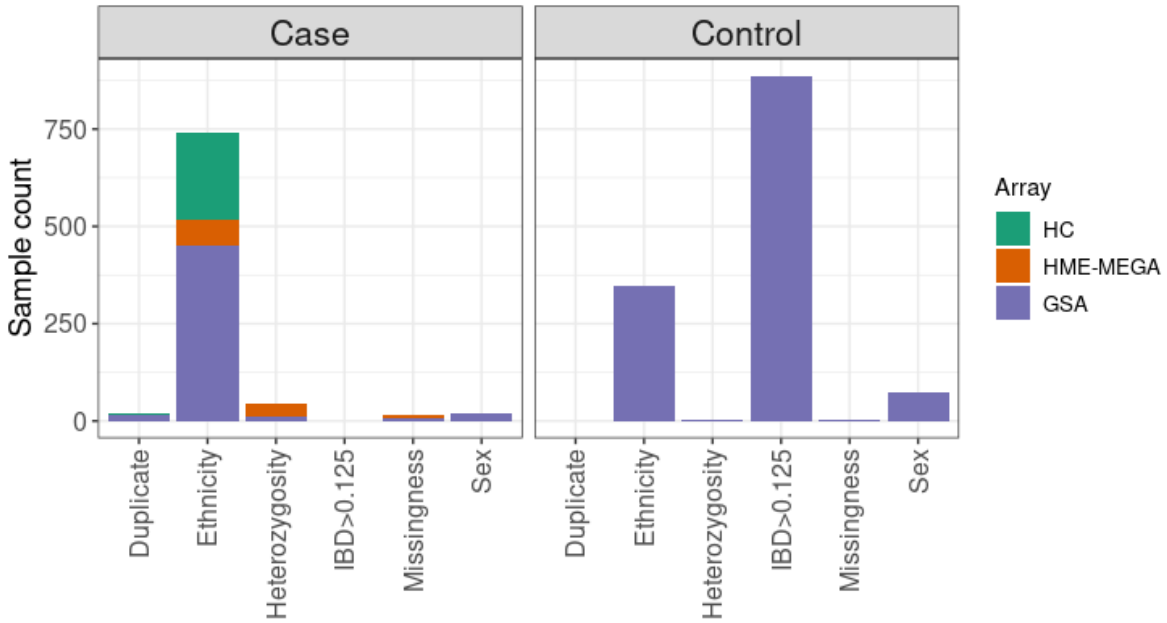
Abbreviations: HC, HumanCore; GSA, Global Screening Array; HME, Human Multi-Ethnic; MEGA, Multi- Ethnic Global Array; QC, quality control; PCA, principal components analysis; EUR, European; HRC, haplotype reference consortium; IBD, identity-by-descent.

Supplementary Figure 3. Ancestry principal components plot for our Case:Control cohort against 1000 Genomes.



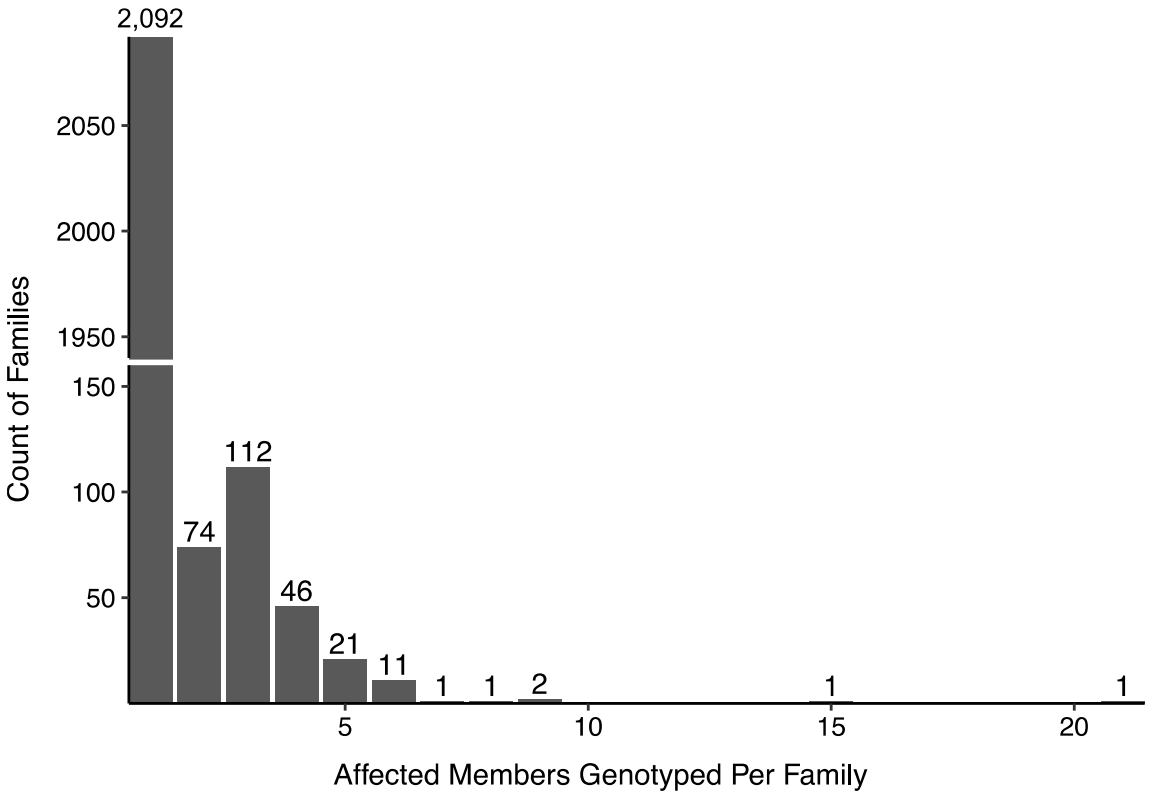
Cases include unaffected relatives.
Abbreviations: PC, principal component; AFR, African; AMR, Admixed; EAS, East Asian; EUR, European; SAS, South Asian.

Supplementary Figure 4. Reason for sample exclusions stratified by Case:Control status.

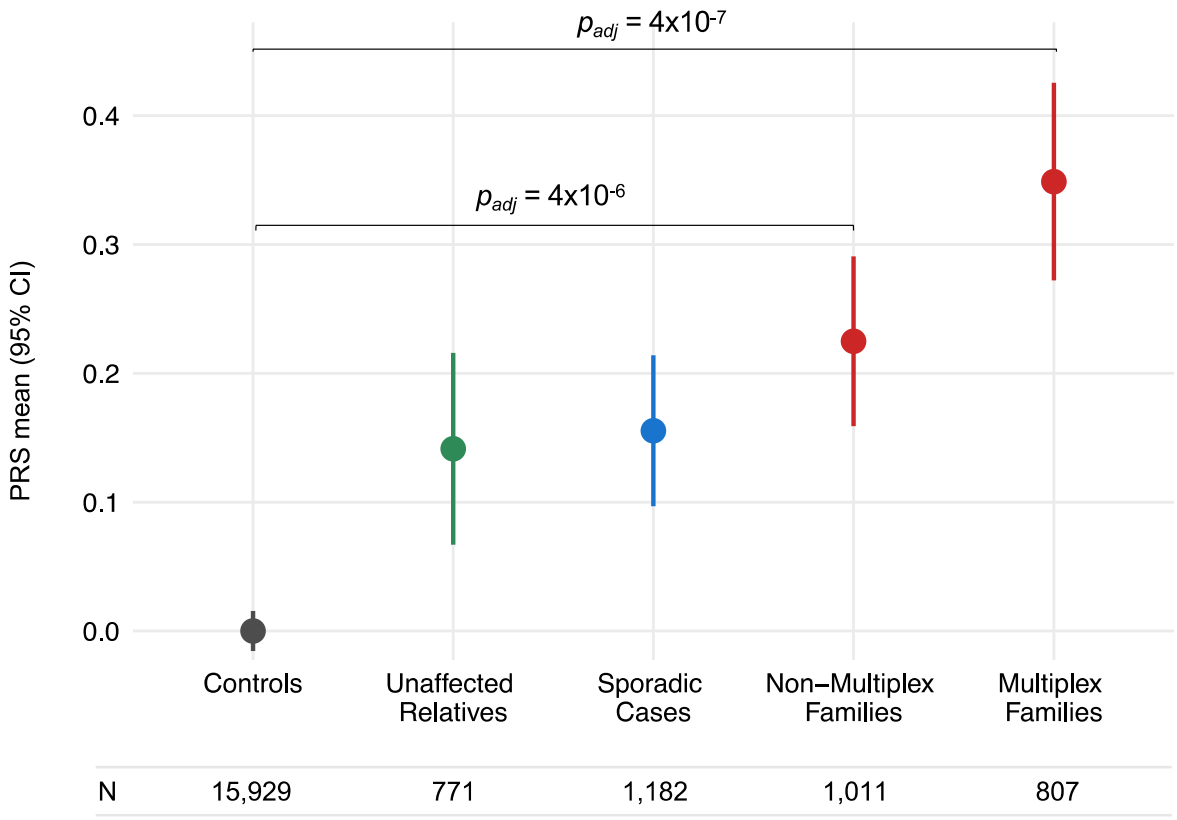


Cases include unaffected relatives.
Abbreviations: HC, HumanCore; GSA, Global Screening Array; HME, Human Multi-Ethnic; MEGA, Multi- Ethnic Global Array; IBD, identity-by-descent estimates.

Supplementary Figure 5. Distribution of affected individuals genotyped per family.
Families of size 1 (n = 2,092) include sporadic cases with no family history of epilepsy (n = 1,182) as well as familial cases where only one affected relative was genotyped (n = 910). An additional 908 familial cases came from 270 families in which two or more affected individuals were genotyped.



Supplementary Figure 6. Epilepsy PRS in multiplex families.
Familial epilepsy cases were further stratified into multiplex families (three or more affected per family) versus the remaining families with only two affected per family or a positive family history with unknown number of affected relatives.



Supplementary Figure 7. PRS in sporadic cases carrying rare variants.
Polygenic risk scores in individuals with sporadic epilepsy who were positive for rare variants (n = 48) versus individuals negative for rare variants (n = 1,134). Statistical comparisons were non-significant ($P_{adj} > 0.05$); see Supplementary Table 9b.