Supplemental Appendix. Optimizing genomic medicine in epilepsy through a gene-customized approach to missense variant interpretation

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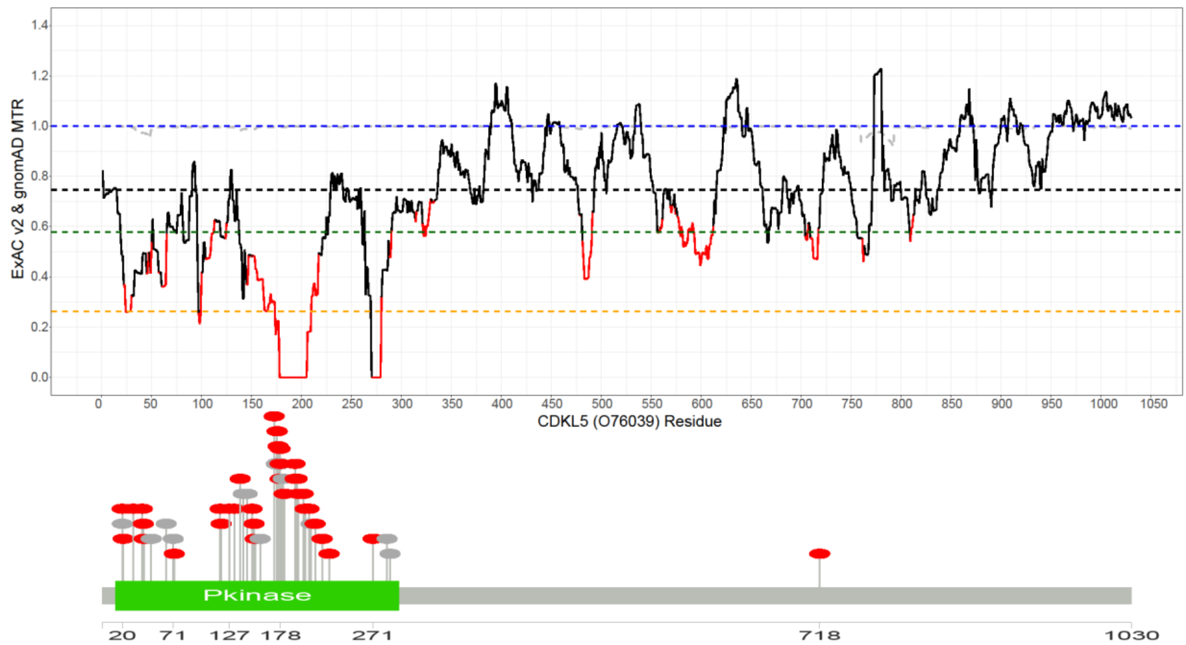
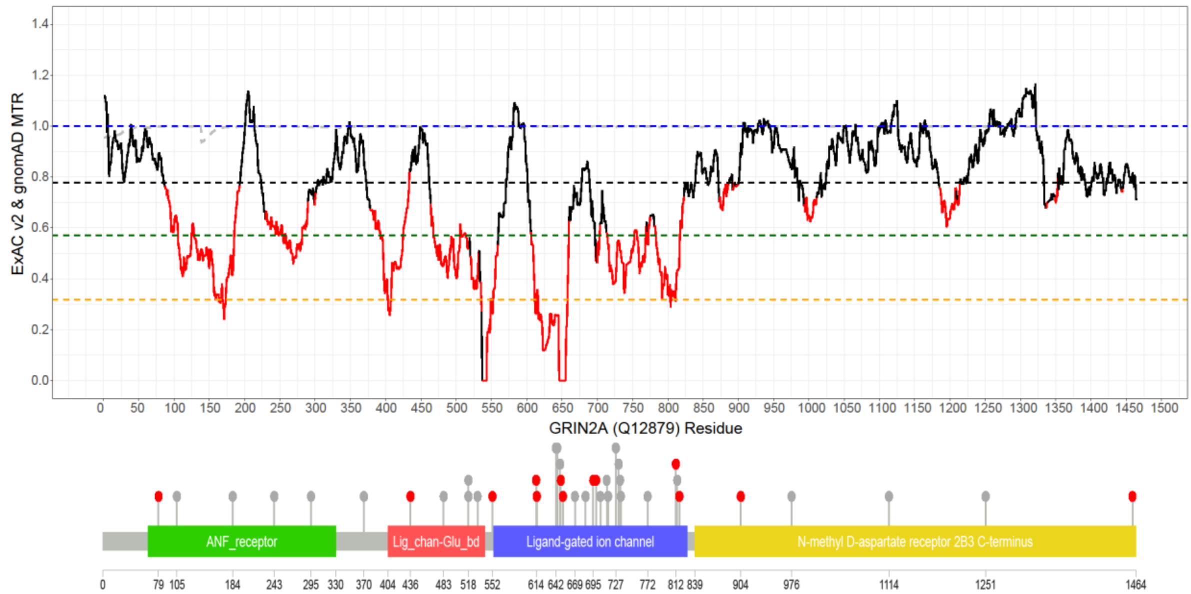
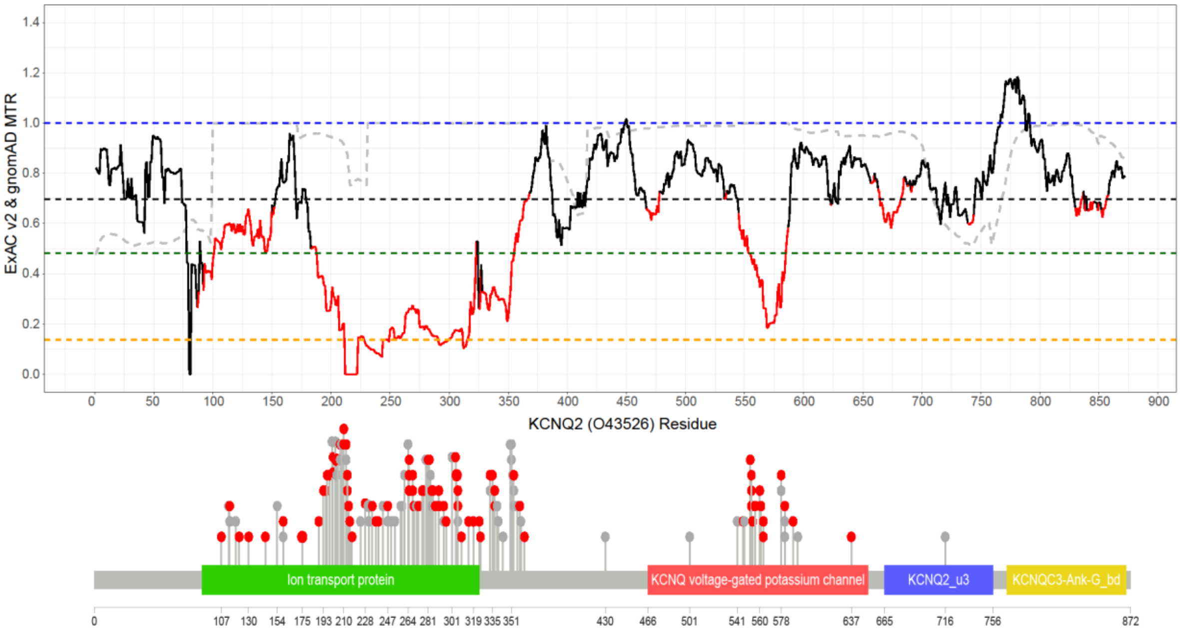
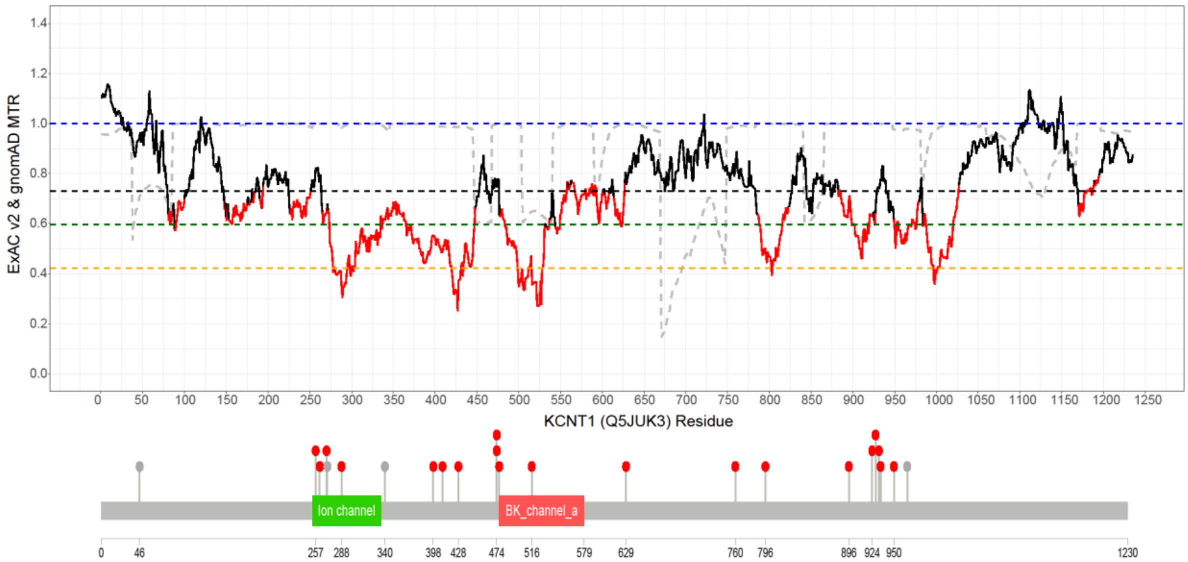
Supplemental Fig. S1: ExAC v2 (release 2.0) MTR plots for the eleven epilepsy genes in the context of pathogenic (qualified and unqualified) missense variant distributions

MTR plots: *CDKL5*, *GRIN2A*, *KCNQ2*, *KCNT1*, *LGI1*, *PCHD19*, *SLC2A1, STXBP1, SCN1A*, *SCN2A*, and *SCN8A*. Regions in red achieved a study-wide FDR < 0.05 (**Supplemental Data S3**). MTR=1 is depicted by dashed blue, the gene's median MTR (dashed black), 25th centile MTR (dashed dark green) and 5th centile MTR (dashed orange). Grey dashed line reflects proportion of all ExAC v2 samples that achieved at least 10-fold coverage at the sites relevant to that codon (Methods). Beneath each MTR plot is the linear distribution of the 606 qualified (red) and 437 unqualified (grey) pathogenic missense variants.

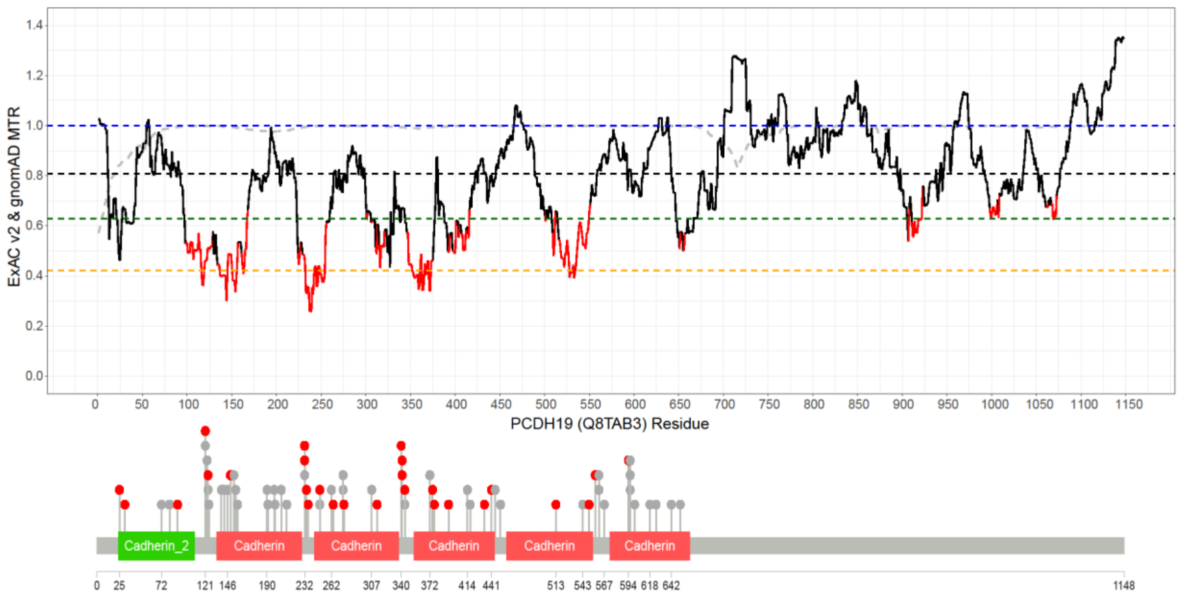


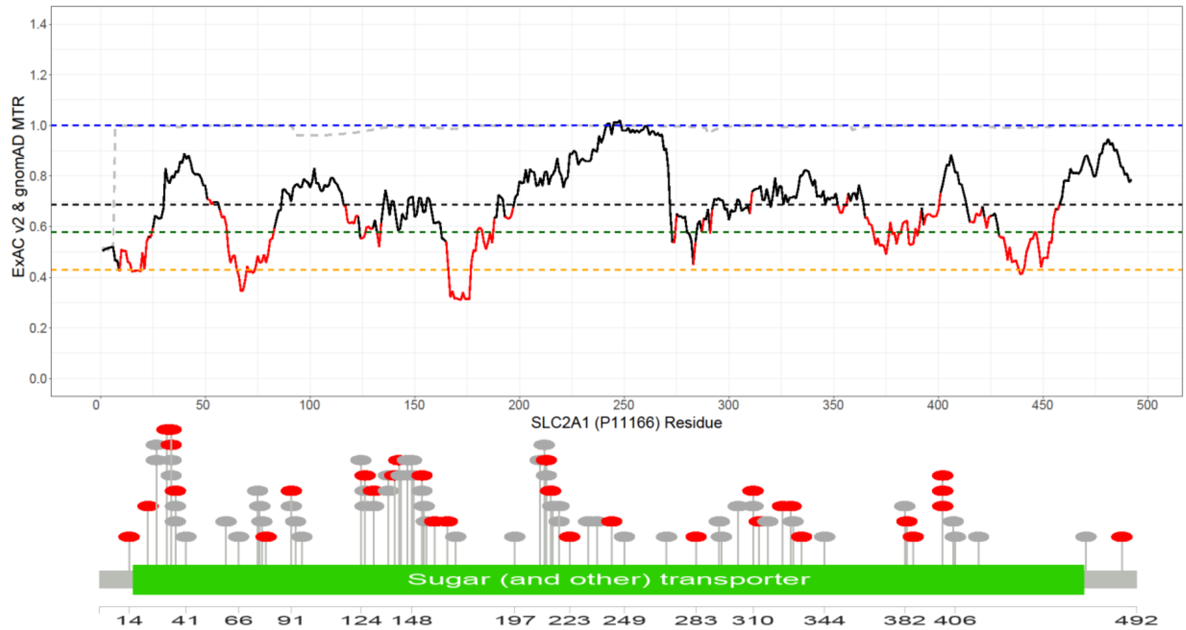


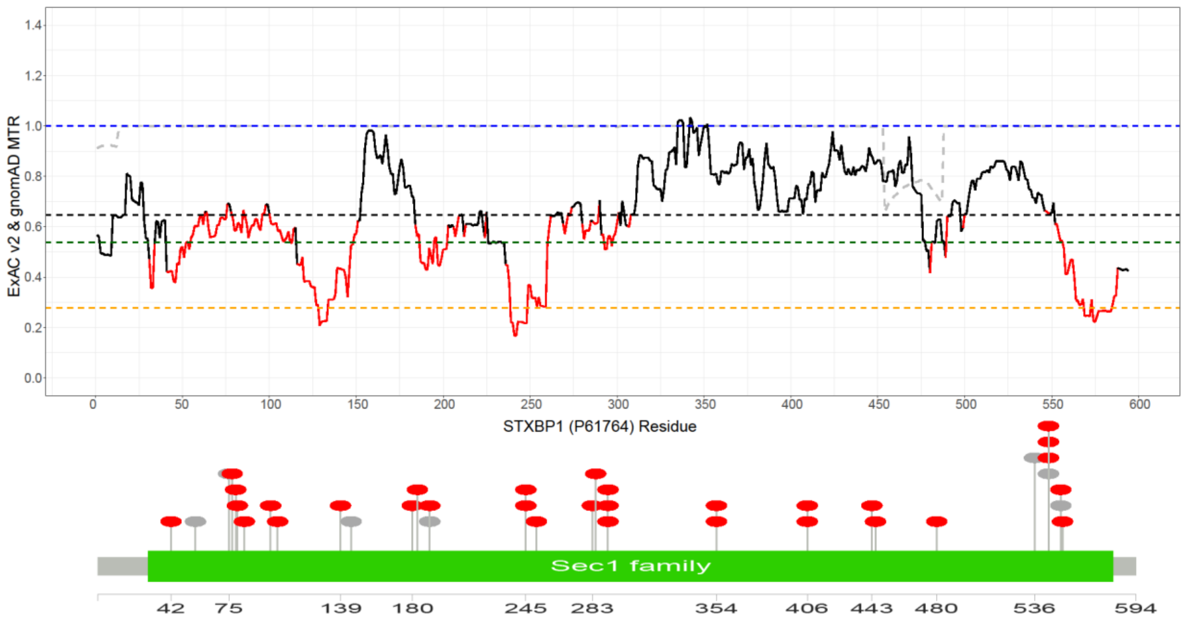

ExAC v2 & gnomAD MTR
KCNQ2 (O43526) Residue
Ion transport protein
KCNQ voltage-gated potassium channel
KCNQ2_u3
KCNQC3-Ank-G_bd

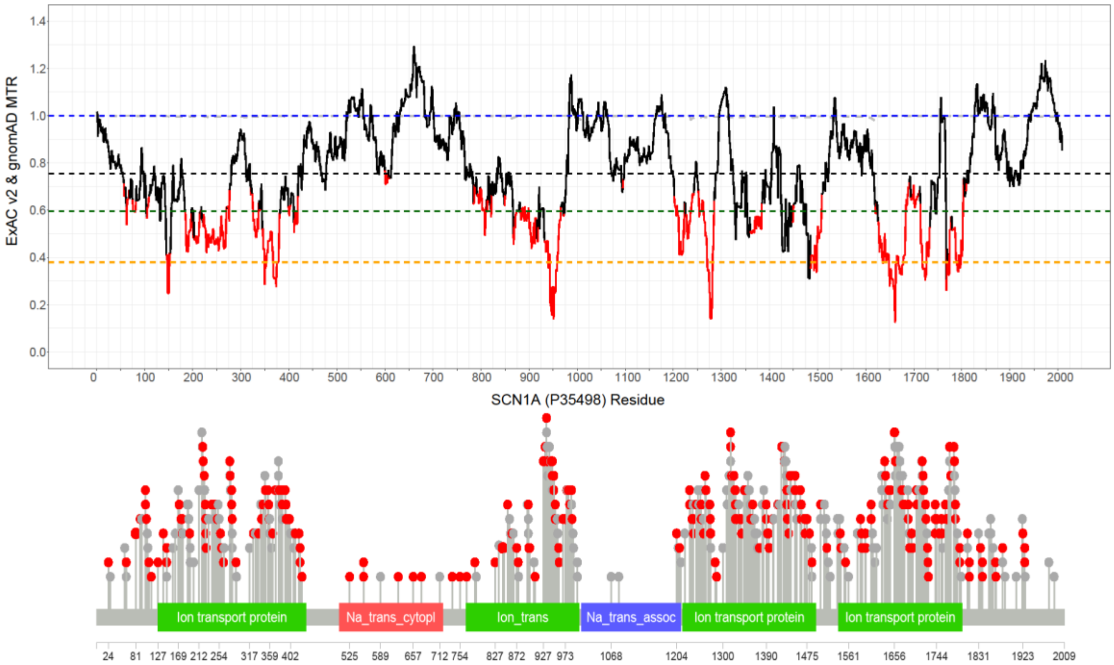

ExAC v2 & gnomAD MTR
KCNT1 (Q5JUK3) Residue
Ion channel
BK_channel_a


ExAC v2 & gnomAD MTR
LGI1 (O95970) Residue
LRR_8
LRR_8
EPTP
EPTP
EPTP
EPTP
EPTP
EPTP
EPTP

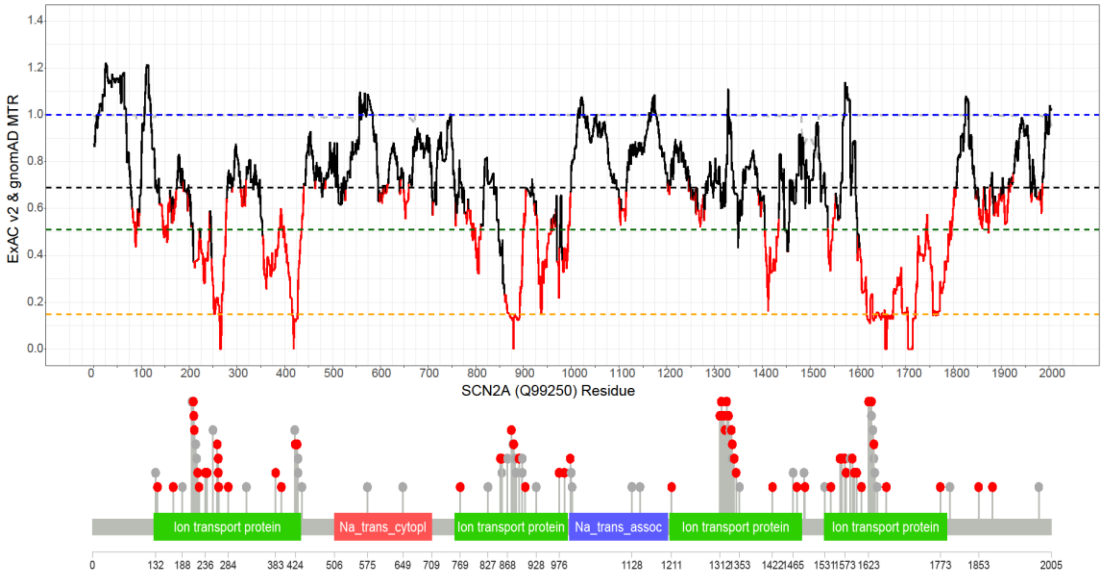

ExAC v2 & gnomAD MTR
PCDH19 (Q8TAB3) Residue
Cadherin_2
Cadherin
Cadherin
Cadherin
Cadherin
Cadherin


ExAC v2 & gnomAD MTR
SLC2A1 (P11166) Residue
Sugar (and other) transporter

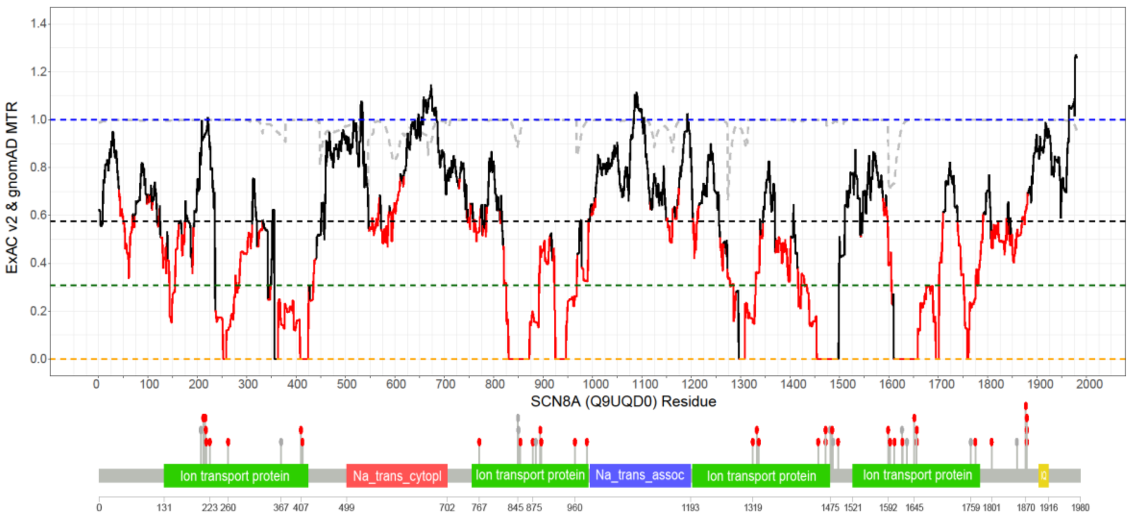

ExAC v2 & gnomAD MTR
STXBP1 (P61764) Residue
Sec1 family



ExAC v2 & gnomAD MTR
SCN1A (P35498) Residue
Ion transport protein
Na_trans_cytopl
Ion_trans
Na_trans_assoc
Ion transport protein
Ion transport protein



ExAC v2 & gnomAD MTR
SCN2A (Q99250) Residue
Ion transport protein
Na_trans_cytopl
Ion transport protein
Na_trans_assoc
Ion transport protein
Ion transport protein



ExAC v2 & gnomAD MTR
SCN8A (Q9UQD0) Residue
Ion transport protein
Na_trans_cytopl
Ion transport protein
Na_trans_assoc
Ion transport protein
Ion transport protein

Supplemental Fig. S2: ExAC v1 (release 0.3.1) MTR plots for the eleven epilepsy genes

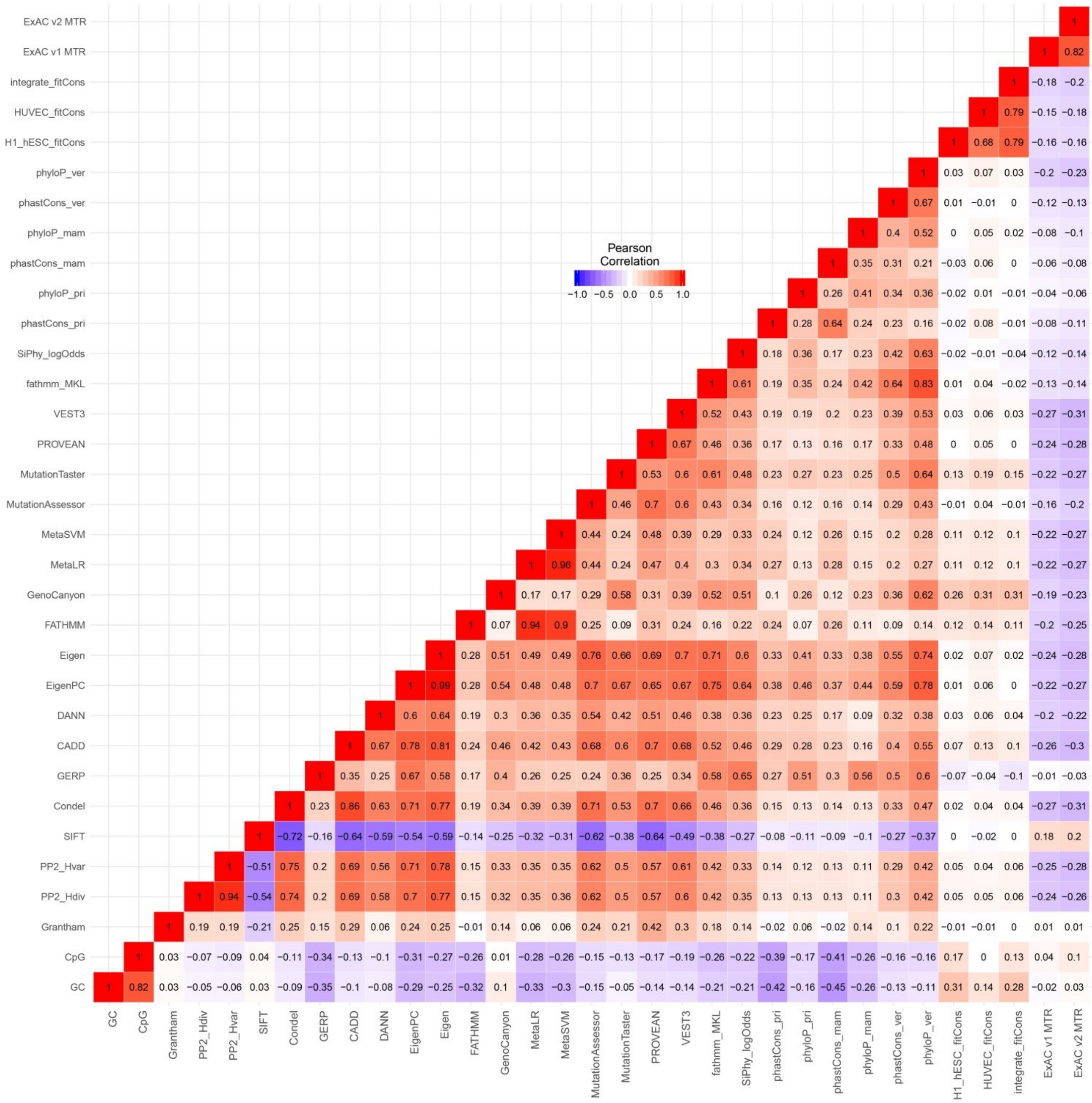
Provided as a separate file – MTR plots for *CDKL5* (**A**), *GRIN2A* (**B**), *KCNQ2* (**C**), *KCNT1* (**D**), *LGI1* (**E**), *PCHD19* (**F**), *SCN1A* (**G**), *SCN2A* (**H**), *SCN8A* (**I**), *SLC2A1* (**J**), and *STXBP1* (**K**). Regions in red achieved a study-wide FDR < 0.05 (**Supplemental Data S3**). MTR = 1 is depicted by the dashed blue line. Multiple gene-specific estimates are also depicted, including a gene's median MTR (black dashed line), 25th percentile MTR (dark green dashed line) and 5th percentile lowest MTR estimates (orange dashed line). The grey dashed line reflects how well that region of the gene was covered in the ExAC v1 sample data by showing the proportion of all ExAC v1 samples that achieved at least 10-fold coverage at the sites relevant to that codon (Methods).

Supplemental Fig. S3: ExAC v1 MTR estimates comparing the pathogenic (red) to the ExAC v2 Control Group 2 (blue) missense variants distributions

Provided as a separate file – presents the qualified pathogenic (red) and the ExAC v2 Control Group 2 (blue) missense variant distributions for the ExAC v1 MTR estimates. Figures include the median MTR estimate for all possible missense variants in the gene (grey dashed line), the 25th percentile MTR estimate (orange dashed line), and the 5th percentile MTR estimate (red dashed line). The position of the median ExAC v1 MTR estimate for the qualified pathogenic variants (red full line) and median for the Control Group 2 (blue full line) are also included. Distributions provided for *CDKL5* (**A**), *GRIN2A* (**B**), *KCNQ2* (**C**), *KCNT1* (**D**), *LGI1* (**E**), *PCHD19* (**F**), *SCN1A* (**G**), *SCN2A* (**H**), *SCN8A* (**I**), *SLC2A1* (**J**), and *STXBP1* (**K**).

Supplemental Fig. S4: Correlation matrix of the evaluated bioinformatic tools (features); including the two additional MTR features

Each cell represents the Pearson's *r* correlation coefficient.



Supplemental Fig. S5: Histogram distribution for all features across all genes

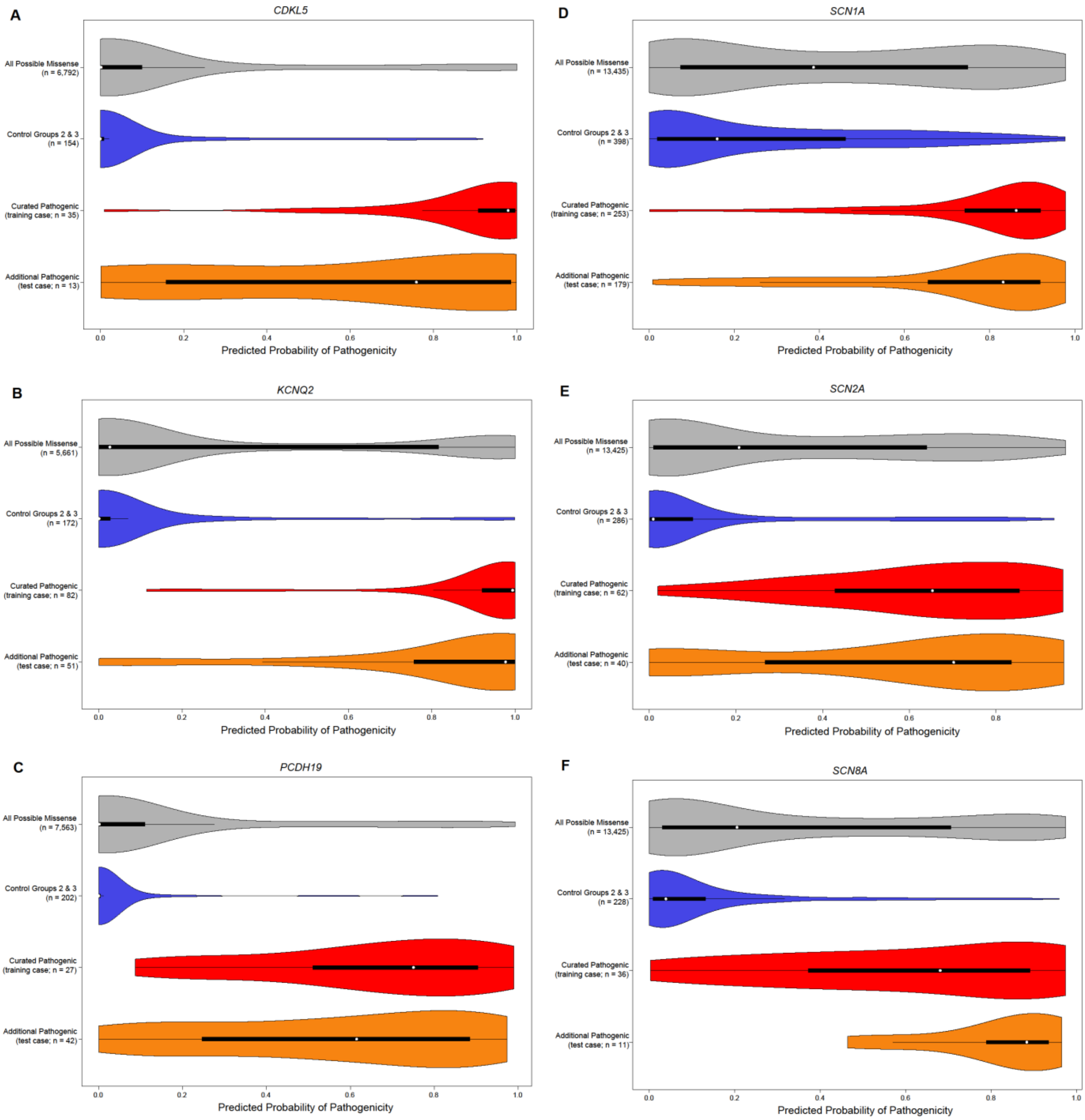
Provided as a separate file – contains the entire set of score distributions for all evaluated bioinformatic tools across each of the eleven evaluated genes. The median rankscore of all possible missense variants (grey line) is illustrated relative to three mutually exclusive control population distributions: Control group 1 (ExAC v1 singletons; blue line), Control group 2 (ExAC v2 singletons not in ExAC v1; dashed blue line) and Control group 3 (ExAC v2 variants with a MAF<0.05% and not in Control groups 1 or 2; dashed light blue line). The median rankscore for two case-ascertained missense variant distributions is also indicated, representing the 606 qualified pathogenic variants (red line), and the additional 437 unqualified pathogenic-reported variants (dashed red line).

Supplemental Fig. S6: Receiver Operating Characteristic (ROC) curves for the six genes with multivariate regression models, including individual features contributing to that model

Provided as a separate file – accompanying the ROC curves for each gene is the AUC for model predictions applied on the training and separately on the test samples that did not contribute to model fitting. ROC curves for *CDKL5* (**A** and **B**), *KCNQ2* (**C** and **D**), *PCHD19* (**E** and **F**), *SCN1A* (**G** and **H**), *SCN2A* (**I** and **J**), and *SCN8A* (**K** and **L**).

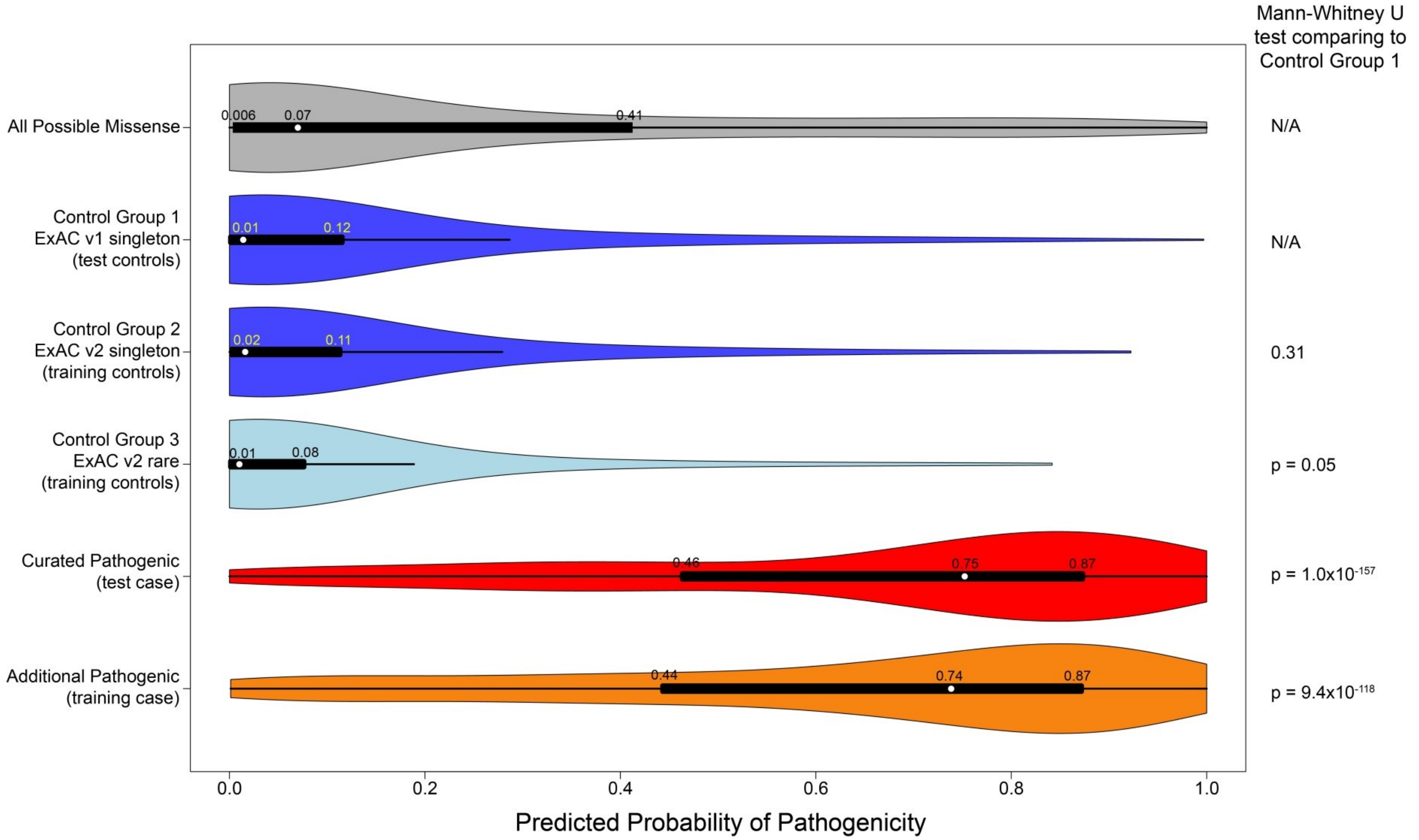
Supplemental Fig. S7: Variant Gene-specific Probability of Pathogenicity (GPP) score distributions, per gene

Violin plots present the distribution of Gene-specific Probability of Pathogenicity (GPP) score for *CDKL5* (**A**), *KCNQ2* (**B**), *PCDH19* (**C**), *SCN1A* (**D**), *SCN2A* (**E**) and *SCN8A* (**F**).



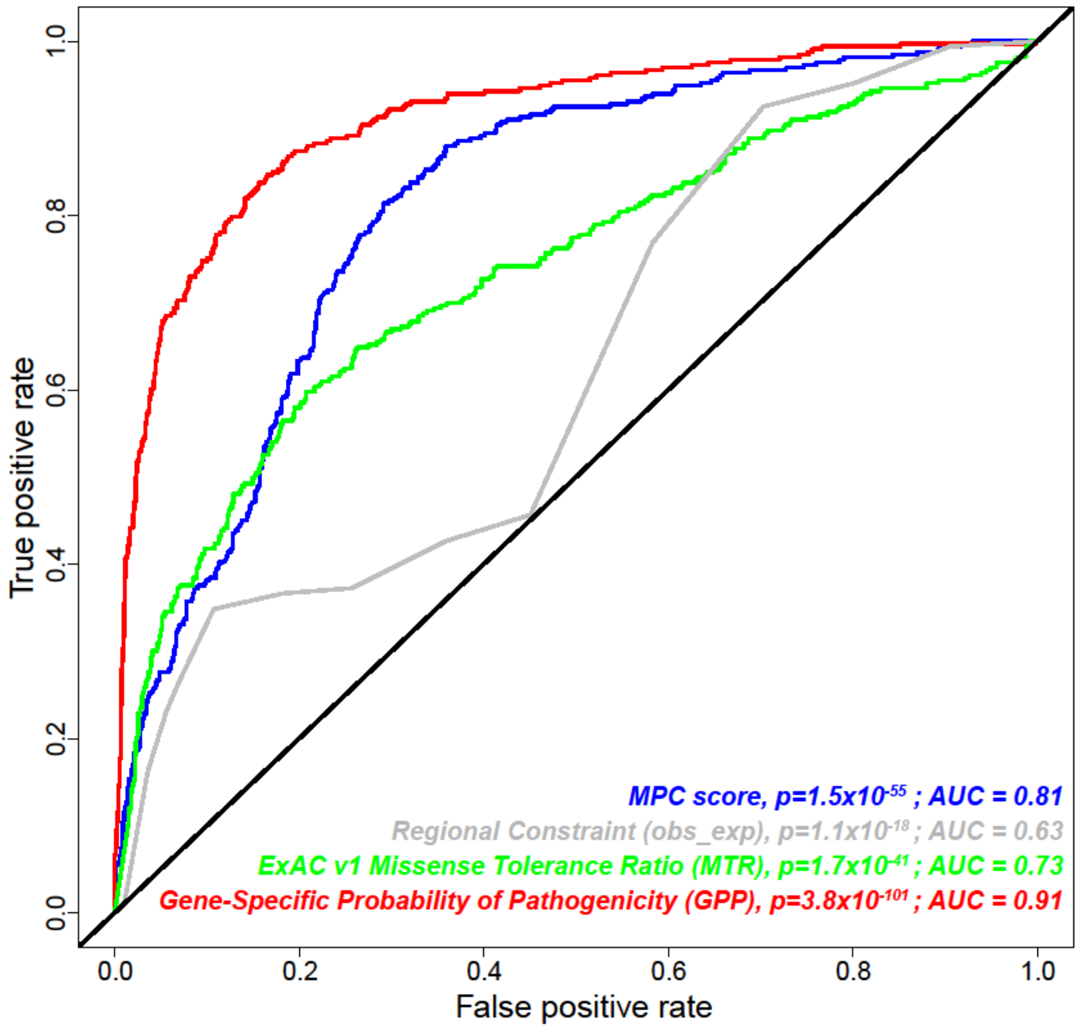
Supplemental Fig. S8: Variant GPP probability of pathogenicity score distribution after fitting the model with the unqualified case variants and the ExAC v2 control missense variants

This plot accompanies Figure 5. It displays the pooled collection of variants from the six genes with a gene-customized model. It differs to Figure 5 by replacing the training data with the test data and recalculating model predictions.



Supplemental Fig. S9: Comparing GPP to MPC predictions for the six epilepsy genes

Samocha et al. (2017 preprint) introduce a new missense variant score, referred to as MPC by the authors (MPC and component scores are available at: ftp.broadinstitute.org/pub/ExAC_release/release1/regional_missense_constraint/). The MPC combines a regional missense constraint score (variable obs_exp) with variant level information (PolyPhen-2 and "missense badness"). For all possible missense variants in the six epilepsy genes with a GPP we found low correlation between MPC and GPP (Pearson's r^2 = 0.329). Directly comparing how well the two scores predict the 336 test-set pathogenic missense variants from the 827 Control Group 2 missense variants found—across these six epilepsy genes—the GPP achieves a superior AUC of 0.91, (95% CI, 0.89 – 0.93) compared to the MPC AUC of 0.81, (95% CI, 0.79 – 0.83); (DeLong's test for two correlated ROC curves p = 4.9×10^{-17}). This evaluation is restricted to the six epilepsy genes with GPP scores.

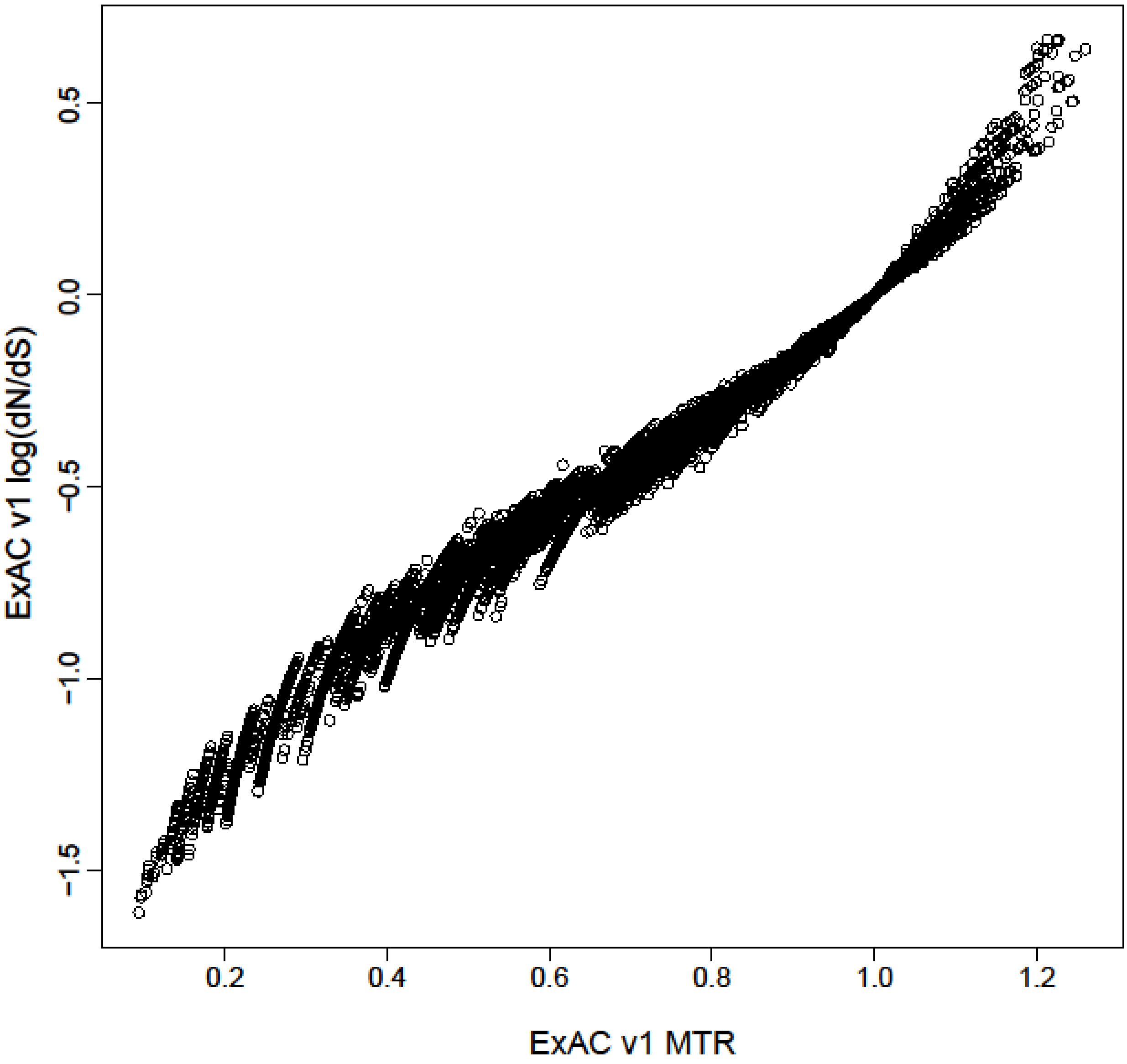


Supplemental Fig. S10: Gene-specific Probability of Pathogenicity (GPP) score distribution after fitting the model with the combined eleven gene data rather than a gene-customized approach

Provided as a separate file. This figure reproduces the model building process applied to individual genes on the pooled collection of data across the eleven epilepsy genes. (**A**) The Boruta assessment evaluating the predictive utility of the individual features in discriminating the 606 qualified pathogenic missense variants from the Control Group 1 variants. (**B**) The forward-backward selection adding features to the model until the AIC suggested that the addition of new features no longer significantly improved the model. (**C**) The resulting logistic regression model for the combined gene (global) fitted model. (**D**) Gene-specific Probability of Pathogenicity (GPP) score distribution after applying the combined-gene (global) model that was fitted to the collection of training variants from the eleven epilepsy genes.

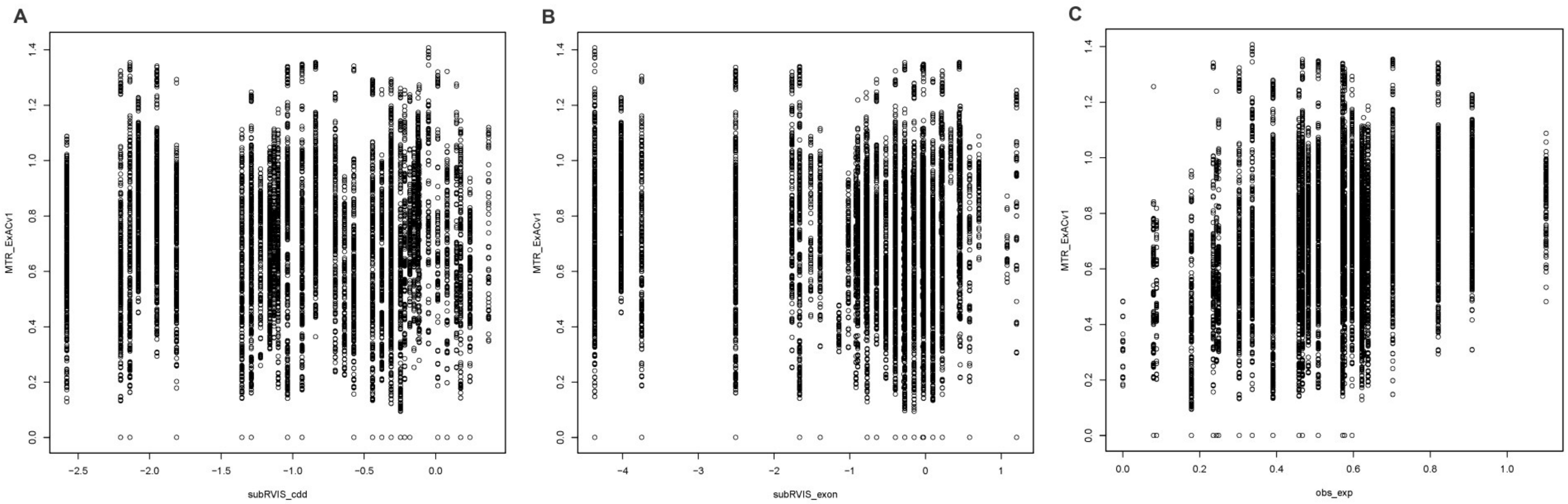
Supplemental Fig. S11: Correlation of MTR with log(dN/dS) using the ExAC v1 sliding windows

The sliding windows from the eleven epilepsy genes were adopted (**Supplemental Data S3**). Due to singularity with $log(d_N/d_S)$ at either $D_n = 0$ or $D_s = 0$, the plot depicts the 92% of windows with $D_n > 0$ and $D_s > 0$.



Supplemental Fig. S12: Comparison of MTR with subRVIS and regional constraint (obs_exp) estimates

Across these 11 epilepsy genes the MTR estimates (Y-axis) are compared to the distribution of **(A)** subRVIS(CDD), **(B)** subRVIS(exon) and **(C)** obs_exp (Samocha et al. [preprint] regional constraint) estimates.



Pearson's $r^2 = 0.008$ Pearson's $r^2 = 0.009$ Pearson's $r^2 = 0.153$

Supplemental Table S1: Breakdown for the curation of the eleven epilepsy genes

Qualification	ClinVar	HGMD	Union (distinct)
Number of variants described in the ClinVar and/or HGMD variant databases	1,570	1,600	2,702
Number of variants classified as pathogenic	631	1,416	1,605
Number of variant effects annotated as "missense" in the canonical transcript	443	988	1,104
Number of variants with an epilepsy-related keyword among the described phenotype	435	939	1,056
Number of variants with "Pathogenic" classification when present among ClinVar and / or HGMD databases (**See Supplemental Table S2**)	422	855	1,043
Final tallies after confirming segregation support based on the associated literature (**See Supplemental Table S2**)	256	531	**606**

Supplemental Table S2: Summary of pathogenic classified variants among eleven epilepsy genes

Gene	HGMD®	ClinVar	Distinct	Qualified Tally [% of overall Distinct]
SCN1A	402	103	**432**	**253 [59%]**
KCNQ2	96	85	**133**	**82 [62%]**
SCN2A	60	63	**102**	**62 [61%]**
SLC2A1	72	26	**84**	**31 [37%]**
PCDH19	55	21	**69**	**27 [39%]**
CDKL5	32	37	**48**	**35 [73%]**
SCN8A	32	26	**47**	**36 [77%]**
GRIN2A	28	20	**39**	**13 [33%]**
STXBP1	28	21	**38**	**31 [82%]**
LGI1	27	10	**27**	**16 [59%]**
KCNT1	23	10	**24**	**20 [83%]**
Total	**855**	**422**	**1,043**	**606 [58%]**

Summarizing the number of pathogenic reported variants among eleven epilepsy genes where at least 20 distinct pathogenic variants were reported among ClinVar and/or HGMD databases.

Supplemental Table S3: Per gene summaries of the standing variation data found among the combined ExAC v2 and gnomAD population sample of 138,632 samples

Gene	CCDS Transcript	CCDS length protein-coding bases	CCDS SNVs protein-coding (% of possible)	Pass Database Quality Filter (% of observed)	Have synonymous or missense effects (% of observed)
CDKL5	CCDS14186.1	3,093	466 (5.0%)	417 (89.5%)	414 (88.8 %)
GRIN2A	CCDS10539.1	4,395	1,175 (8.9%)	1,070 (91.1%)	1,064 (90.6%)
KCNQ2	CCDS13520.1	2,619	765 (9.7%)	509 (66.5%)	508 (66.4%)
KCNT1	CCDS35175.2	3,708	1,330 (12.0%)	985 (74.1%)	974 (73.2%)
LGI1	CCDS7431.1	1,674	303 (6.0%)	275 (90.8%)	273 (90.1%)
PCDH19	CCDS55462.1	3,447	647 (6.3%)	535 (82.7%)	535 (82.7%)
SCN1A	CCDS54413.1	6,030	1,088 (6.0%)	1,020 (93.8%)	1,018 (93.6%)
SCN2A	CCDS33314.1	6,018	988 (5.5%)	871 (88.2%)	869 (88.0%)
SCN8A	CCDS44891.1	5,943	951 (5.3%)	789 (83.0%)	786 (82.6%)
SLC2A1	CCDS477.1	1,479	359 (8.1%)	299 (83.3%)	299 (83.3%)
STXBP1	CCDS35146.1	1,785	306 (5.7%)	260 (85.0%)	260 (85.0%)

Supplemental Table S4: List of the 31 tools (predictors / features) evaluated on the eleven genes

Database Source	Description	Feature ID in this paper
PolyPhen-2	PolyPhen-2 HumDiv score	PP2_Hdiv
PolyPhen-2	PolyPhen-2 HumVar score	PP2_Hvar
Ve!P Ensembl r87	SIFT score	SIFT
Ve!P Ensembl r87	Condel score	Condel
CADD v1.3	GC (%GC in a window of +/-75bp)	GC
CADD v1.3	CpG (%CpG in a window of +/-75bp)	CpG
CADD v1.3	Grantham score	Grantham
CADD v1.3	phastCons primate score	phastCons_pri
CADD v1.3	phyloP primate score	phyloP_pri
dbNSFP 3.2c	GERP++ RS rankscore	GERP_rs
dbNSFP 3.2c	CADD raw rankscore	CADD_rs
dbNSFP 3.2c	DANN rankscore	DANN_rs
dbNSFP 3.2c	Eigen-PC-raw rankscore^	EigenPC_rs
dbNSFP 3.2c	Eigen-raw rankscore^	Eigen_rs
dbNSFP 3.2c	FATHMM converted rankscore	FATHMM_rs
dbNSFP 3.2c	GenoCanyon score rankscore	GenoCanyon_rs
dbNSFP 3.2c	MetaLR rankscore	MetaLR_rs
dbNSFP 3.2c	MetaSVM rankscore	MetaSVM_rs
dbNSFP 3.2c	MutationAssessor score rankscore	MutationAssessor_rs
dbNSFP 3.2c	MutationTaster converted rankscore	MutationTaster_rs
dbNSFP 3.2c	PROVEAN converted rankscore	PROVEAN_rs
dbNSFP 3.2c	VEST 3.0 rankscore	VEST3_rs
dbNSFP 3.2c	fathmm-MKL coding rankscore	fathmm_MKL_rs
dbNSFP 3.2c	SiPhy 29way logOdds rankscore	SiPhy_logOdds_rs
dbNSFP 3.2c	phastCons20way mammalian rankscore	phastCons_mam_rs
dbNSFP 3.2c	phyloP20way mammalian rankscore	phyloP_mam_rs
dbNSFP 3.2c	phastCons100way vertebrate rankscore	phastCons_ver_rs
dbNSFP 3.2c	phyloP100way vertebrate rankscore	phyloP_ver_rs
dbNSFP 3.2c	H1-hESC fitCons score rankscore^	H1_hESC_fitCons_rs
dbNSFP 3.2c	HUVEC fitCons score rankscore^	HUVEC_fitCons_rs
dbNSFP 3.2c	integrated fitCons score rankscore^	integrate_fitCons_rs

^Scores were unavailable for these five tools on the X chromosome genes *CDKL5* and *PCDH19*.

Supplemental Table S5: List of the features found by the Boruta algorithm to be uninformative, informative/tentative, and highly informative for each studied gene

Gene	Uninformative	Informative / Tentative	Highly Informative
CDKL5	15. phastCons 16. GenoCanyon 17. Grantham 18. phyloP_mam	7. GC 8. PP2_Hdiv 9. SIFT 10. phyloP_pri 11. DANN 12. SiPhy_logOdds 13. GERP 14. fathmm_MKL	**1. VEST3** **2. MutationAssessor** **3. PROVEAN** 4. MutationTaster 5. CADD 6. FATHMM
GRIN2A	10. H1_hESC_fitCons 11. HUVEC_fitCons 12. GERP 13. PP2_Hdiv 14. FATHMM 15. phyloP_pri 16. fathmm_MKL 17. MutationTaster 18. Grantham 19. phyloP_mam 20. GC	1. CADD 2. SIFT 3. VEST3 4. MutationAssessor 5. PROVEAN 6. GenoCanyon 7. SiPhy_logOdds 8. phastCons 9. DANN	
KCNQ2	19. phastCons 20. Grantham	9. GC 10. CADD 11. PP2_Hdiv 12. FATHMM 13. SiPhy_logOdds 14. GenoCanyon 15. phyloP_pri 16. DANN 17. fathmm_MKL 18. phyloP_mam	**1. VEST3** **2. PROVEAN** **3. MutationAssessor** 4. H1_hESC_fitCons 5. HUVEC_fitCons 6. SIFT 7. GERP 8. MutationTaster
KCNT1	11. HUVEC_fitCons 12. FATHMM 13. GenoCanyon 14. phyloP_pri 15. H1_hESC_fitCons 16. phyloP_mam 17. fathmm_MKL 18. phastCons 19. Grantham 20. GC	1. VEST3 2. DANN 3. CADD 4. MutationAssessor 5. SIFT 6. PP2_Hdiv 7. PROVEAN 8. MutationTaster 9. SiPhy_logOdds 10. GERP	
LGI1	8. SiPhy_logOdds 9. DANN 10. GenoCanyon 11. MutationTaster 12. H1_hESC_fitCons 13. phastCons 14. phyloP_pri 15. GERP 16. GC	6. CADD 7. MutationAssessor	**1. VEST3** 2. SIFT **3. PROVEAN** 4. PP2_Hdiv 5. Grantham

	17. fathmm_MKL 18. FATHMM 19. phyloP_mam 20. HUVEC_fitCons		
PCDH19	9. GC 10. MutationTaster 11. Grantham 12. DANN 13. fathmm_MKL 14. phyloP_pri 15. GenoCanyon 16. phyloP_mam 17. phastCons 18. SiPhy_logOdds	4. FATHMM 5. SIFT 6. PP2_Hdiv 7. CADD 8. GERP	**1. MutationAssessor** **2. VEST3** 3. **PROVEAN**
SCN1A	15. DANN 16. phyloP_pri 17. HUVEC_fitCons 18. GC 19. phyloP_mam 20. phastCons	10. GenoCanyon 11. GERP 12. H1_hESC_fitCons 13. SiPhy_logOdds 14. Grantham	**1. VEST3** 2. FATHMM **3. MutationAssessor** 4. CADD 5. SIFT **6. PROVEAN** 7. MutationTaster 8. PP2_Hdiv 9. fathmm_MKL
SCN2A	14. phyloP_mam 15. SiPhy_logOdds 16. phyloP_pri 17. Grantham 18. phastCons 19. GC 20. H1_hESC_fitCons	5. fathmm_MKL 6. SIFT 7. MutationTaster 8. DANN 9. GERP 10. CADD 11. PROVEAN 12. HUVEC_fitCons 13. GenoCanyon	1. FATHMM **2. VEST3** 3. PP2_Hdiv **4. MutationAssessor**
SCN8A	13. DANN 14. MutationTaster 15. SiPhy_logOdds 16. GenoCanyon 17. phyloP_mam 18. fathmm_MKL 19. Grantham 20. HUVEC_fitCons	3. FATHMM 4. SIFT 5. CADD 6. MutationAssessor 7. PROVEAN 8. H1_hESC_fitCons 9. PP2_Hdiv 10. phyloP_pri 11. GERP 12. GC	**1. VEST3** 2. phastCons
SLC2A1	11. fathmm_MKL 12. SiPhy_logOdds 13. GERP 14. H1_hESC_fitCons 15. phastCons 16. FATHMM 17. GenoCanyon 18. HUVEC_fitCons 19. phyloP_pri 20. GC	2. CADD 3. PP2_Hdiv 4. VEST3 5. SIFT 6. phyloP_mam 7. PROVEAN 8. Grantham 9. MutationTaster 10. DANN	**1. MutationAssessor**
STXBP1	9. Grantham 10. SiPhy_logOdds	5. FATHMM 6. PP2_Hdiv	**1. VEST3** **2. MutationAssessor**

	11. CADD 12. GERP 13. phastCons 14. DANN 15. phyloP_pri 16. GC 17. H1_hESC_fitCons 18. phyloP_mam 19. HUVEC_fitCons 20. GenoCanyon	7. fathmm_MKL 8. MutationTaster	**3. PROVEAN** 4. SIFT

Within each category the tools are further sorted from highest to lowest median z-scores.

Supplemental Table S6: Summary of the top feature distribution for the nine epilepsy genes with at least one highly informative feature

Gene	Top-ranked feature	Gene-wide	Control Group 1: ExACv1 (training)	Control Group 2: ExACv2 (test)	Control Group 3: ExACv2 (MAF<0.05)	Qual. Case (training)	Non-Qual Case (test)	MWU p-value (*ExACv2 vs Non-Qual Case*)	MWU p-value (*ExACv2 vs ExACv1*)
CDKL5	VEST3	6792; 0.504 [0.24 - 0.73]	104; 0.302 [0.11 - 0.63]	79; 0.336 [0.14 - 0.68]	75; 0.221 [0.09 - 0.55]	35; 0.973 [0.89 - 0.99]	13; 0.963 [0.93 - 0.99]	$1.4x10^{-6}$	0.29
KCNQ2	VEST3	5661; 0.668 [0.45 - 0.83]	98; 0.616 [0.43 - 0.73]	89; 0.535 [0.30 - 0.72]	83; 0.541 [0.28 - 0.72]	82; 0.892 [0.81 - 0.95]	51; 0.853 [0.75 - 0.93]	$9.4x10^{-12}$	0.22
LGI1	VEST3	3690; 0.697 [0.47 - 0.85]	67; 0.489 [0.29 - 0.68]	53; 0.528 [0.33 - 0.71]	40; 0.468 [0.33 - 0.69]	16; 0.972 [0.96 - 0.99]	11; 0.982 [0.97 - 0.99]	$1.4x10^{-6}$	0.58
PCDH19	Mutation Assessor	7563; 0.323 [0.13 - 0.72]	125; 0.298 [0.13 - 0.67]	117; 0.251 [0.13 - 0.50]	85; 0.225 [0.11 - 0.50]	27; 0.982 [0.92 - 0.99]	42; 0.972 [0.92 - 0.99]	$3.3x10^{-17}$	0.07
SCN1A	VEST3	13435; 0.74 [0.53 - 0.89]	207; 0.596 [0.34 - 0.79]	229; 0.630 [0.41 - 0.79]	169; 0.625 [0.31 - 0.77]	253; 0.965 [0.91 - 0.99]	179; 0.944 [0.90 - 0.98]	$6.3x10^{-39}$	0.40
SCN2A	FATHMM	13425; 0.97 [0.96 - 0.98]	190; 0.962 [0.91 - 0.97]	188; 0.964 [0.91 - 0.97]	98; 0.961 [0.91 - 0.97]	62; 0.984 [0.98 - 0.99]	40; 0.984 [0.97 - 0.99]	$8.6x10^{-7}$	0.48
SCN8A	VEST3	13245; 0.74 [0.52 - 0.88]	167; 0.586 [0.35 - 0.73]	125; 0.562 [0.36 - 0.77]	103; 0.560 [0.37 - 0.71]	36; 0.821 [0.74 - 0.93]	11; 0.937 [0.89 - 0.95]	$1.1x10^{-5}$	0.69
SLC2A1	Mutation Assessor	3188; 0.670 [0.34 - 0.92]	50; 0.403 [0.21 - 0.80]	52; 0.426 [0.19 - 0.69]	51; 0.463 [0.22 - 0.73]	31; 0.973 [0.89 - 0.99]	53; 0.943 [0.76 - 0.98]	$2.9x10^{-10}$	0.52
STXBP1	VEST3	3939; 0.733 [0.55 - 0.87]	46; 0.592 [0.47 - 0.74]	55; 0.689 [0.39 - 0.78]	27; 0.627 [0.31 - 0.73]	31; 0.975 [0.96 - 0.99]	7; 0.945 [0.84 - 0.95]	$3.6x10^{-10}$	0.60

The individual cells represent the total number of variants; the median rankscore of the corresponding variant distribution accompanied by the lower and upper quantile estimates of their distribution [Q1 - Q3]. MWU: Mann-Whitney U test

Supplemental Table S7: Akaike information criterion (AIC) comparisons across growing logistic regression models, per gene

Gene	Data	Feature 1	Feature 2	Feature 3	Feature 4	Feature 5	Feature 6
CDKL5	**Feature**	**MTR ExAC v1**	**VEST3**	**Mutation Assessor**	PROVEAN		
	AIC	**100.36**	**44.537**	**35.657**	34.433		
	p-value	**N/A**	**p=7.6x10^{-13}**	**p=0.012**	p=0.542		
GRIN2A	**Feature**	**CADD**	SIFT				
	AIC	**92.783**	87.234				
	p-value	**N/A**	p=0.062				
KCNQ2	**Feature**	**MTR ExAC v1**	**VEST3**	**PROVEAN**	**Mutation Assessor**	H1 hESC fitCons	
	AIC	**126.01**	**81.641**	**68.426**	**56.827**	56.353	
	p-value	**N/A**	**p=2.3x10^{-10}**	**p=0.001**	**p=0.003**	p=0.79	
KCNT1	**Feature**	**VEST3**	DANN				
	AIC	**122.49**	120.19				
	p-value	**N/A**	p=0.32				
LGI1	**Feature**	**VEST3**	SIFT				
	AIC	**21.271**	17.639				
	p-value	**N/A**	p=0.16				
PCDH19	**Feature**	**MTR ExAC v1**	**Mutation Assessor**	**VEST3**	**PROVEAN**	FATHMM	
	AIC	**136.55**	**90.366**	**81.272**	**67.384**	68.077	
	p-value	**N/A**	**p=9.4x10^{-11}**	**p=0.011**	**p=9.6x10^{-4}**	P=1	
SCN1A	**Feature**	**MTR ExAC v1**	**VEST3**	FATHMM			
	AIC	**533.26**	**348.37**	346.03			
	p-value	**N/A**	**p=7.1x10^{-41}**	p=0.31			
SCN2A	**Feature**	**MTR ExAC v1**	**FATHMM**	**VEST3**	**PolyPhen-2 HumDiv**	Mutation Assessor	
	AIC	**223.77**	**183.27**	**160.92**	**152.08**	153.47	
	p-value	**N/A**	**p=1.6x10^{-9}**	**p=1.4x10^{-5}**	**p=0.012**	p=1	
SCN8A	**Feature**	**MTR ExAC v1**	**VEST3**	phastCons primate			
	AIC	**126.23**	**104.68**	103.12			
	p-value	**N/A**	**p=2.1x10^{-5}**	p=0.46			
SLC2A1	**Feature**	**Mutation Assessor**	CADD				
	AIC	**84.088**	82.522				
	p-value	**N/A**	p=0.46				
STXBP1	**Feature**	**VEST3**	Mutation Assessor				
	AIC	**36.27**	33.695				
	p-value	**N/A**	p=0.28				

AIC = Akaike information criterion; MTR = Missense Tolerance Ratio

Supplemental Table S8: Final multivariate logistic regression models, per gene.

Gene	Data	Intercept	X_1	X_2	X_3	X_4	X_5
CDKL5	Feature	N/A	MTR ExAC v1	VEST3	Mutation Assessor		
	β	-11.72	-5.66	13.35	7.35		
	p-value	p=0.0065	p=0.028	$p=6.4x10^{-4}$	p=0.016		
KCNQ2	Feature	N/A	MTR ExAC v1	VEST3	PROVEAN	Mutation Assessor	
	β	-9.70	-11.65	7.64	6.31	8.40	
	p-value	$p=2.7x10^{-4}$	$p=4.7x10^{-7}$	p=0.003	p=0.01	$p=6.6x10^{-4}$	
PCDH19	Feature	N/A	MTR ExAC v1	Mutation Assessor	VEST3	PROVEAN	
	β	-11.81	-3.77	3.27	4.84	9.62	
	p-value	$p=5.7x10^{-5}$	p=0.008	p=0.05	p=0.03	p=0.002	
SCN1A	Feature	N/A	MTR ExAC v1	VEST3			
	β	-5.16	-2.69	8.98			
	p-value	$p=2.7x10^{-8}$	$p=1.3x10^{-6}$	$p=3.3x10^{-20}$			
SCN2A	Feature	N/A	MTR ExAC v1	FATHMM	VEST3	PolyPhen-2 HumDiv	
	β	-35.33	-2.97	31.98	3.68	3.00	
	p-value	p=0.02	$p=1.9x10^{-4}$	p=0.04	p=0.002	p=0.02	
SCN8A	Feature	N/A	MTR ExAC v1	VEST3			
	β	-3.25	-5.40	6.91			
	p-value	p=0.01	$p=2.8x10^{-8}$	$p=1.3x10^{-4}$			

P-values for each X_i tests the null hypothesis that the coefficient of that feature has no effect.
MTR = Missense Tolerance Ratio.

Supplemental Data S1: Review of segregation support accompanying missense variants reported in the eleven genes among ClinVar and/or HGMD® catalogs

Separate data file submission.

Supplemental Data S2: Summary of patient-ascertained pathogenic missense variants found to overlap with population reference cohorts

Separate data file submission.

Supplemental Data S3: ExAC v1 and ExAC v2 MTR estimates for all windows across the eleven epilepsy genes

Separate data file submission.

Supplemental Data S4: Contains the full set of annotations for every possible missense variant in the canonical transcript of each of the eleven epilepsy genes

Separate data file submission.