Application of Rare Variant Transmission-Disequilibrium Tests to Epileptic Encephalopathy Trio Sequence Data

Running title: Epileptic encephalopathies and recessive genotypes

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Abstract

The classic epileptic encephalopathies, including Infantile Spasms (IS) and Lennox-Gastaut Syndrome (LGS), are severe seizure disorders that usually arise sporadically. *De novo* mutations in genes mainly encoding ion channel and synaptic proteins have been found to account for over 15% of patients with IS or LGS. The contribution of autosomal recessive genetic variation, however, is less well understood. We implemented a rare variant transmission-disequilibrium test (TDT) to search for autosomal recessive epileptic encephalopathy genes in a cohort of 320 outbred patient-parent trios that were generally pre-screened for rare metabolic disorders. In the current sample, our rare variant transmission-disequilibrium test did not identify individual genes with significantly distorted transmission over expectation after correcting for the multiple tests. While the rare variant transmission-disequilibrium test did not find evidence of a role for autosomal recessive genes, our current sample is insufficiently powered to assess the overall role of autosomal recessive genotypes in an outbred epileptic encephalopathy population.

Keywords: Epileptic encephalpathy; infantile spasms; transmission-disequilibrium test (TDT); recessive genotypes

INTRODUCTION

Epileptic encephalopathies are severe and therapy-resistant epilepsies of childhood, which frequently lead to developmental delay and multiple associated medical issues. Infantile Spasms (IS) and Lennox-Gastaut Syndrome (LGS) represent two of the more common broad subtypes of epileptic encephalopathies. Many novel genes for epileptic encephalopathies have been discovered in the last five years, fueled by the access to whole-exome sequencing. In particular, exome sequencing has highlighted the important role of *de novo* mutations with current estimates suggesting that over 15% of classical epileptic encephalopathy cases are explained by a *de novo* mutation in an established epileptic encephalopathy gene.[1; 2] Up to a further 3% have been reported to be explained by likely pathogenic *de novo* copy number variants (CNVs).[3]

While the role of *de novo* genetic variation in epileptic encephalopathies is increasingly understood, the role of recessive genetic variation, outside of recessive neurometabolic disorders such as lysosomal disorders, amino acid or organic acid imbalances, congenital disorders of glycosylation, and some mitochondrial diseases, remains unclear. In our current study we systematically assessed autosomal recessive inheritance in 320 IS or LGS patient-parent trios who did not have a likely disease-causing *de novo* mutation among one of the established dominant epileptic encephalopathy genes.[1; 2] In general, the 320 cases studied here had already been intensively studied for neurometabolic disorders using biochemical assessments.

SUBJECTS and METHODS

Cohort

Three-hundred and twenty epileptic encephalopathy trios were recruited through multiple international consortia, including 57 IS or LGS trios unpublished in our earlier studies.[1; 2] Patients did not have a clearly identified metabolic or genetic cause for their epilepsy based on clinically available testing, which varied across institutions. This collection of 320 trios did not include: a) patients previously found to have a *de novo* mutation in an established dominant epileptic encephalopathy gene, and b) trios where exome sequencing was based on a lymphoblastoid cell line (LCL) source for at least one of the three family members. The overall cohort was not enriched for consanguineous parents. Only two parent pairs showed an identity-by-descent (IBD) > 0.125, both < 0.15, which is approximately equivalent to 3rd degree relatives.[4]

Among the 320 trios; two families reported multiple affected children. For one of these families both the proband and affected sibling were investigated through exome sequencing while for the second family only the proband and parents were studied. Sequencing methods used to generate the sequence data have been previously described.[1; 2]

Transmission Disequilibrium Tests

For the transmission test we used two approaches that we have previously introduced.[5; 6] First, we tested for an autosomal homozygous or compound heterozygous effect using coreTDT.[6] In computing the test, we selected loss-of-function and missense single nucleotide substitution variants (SNVs) found at a global population minor allele frequency

below five percent (MAF<0.05). The loss-of-function variants were defined as stop gain, stop lost, start lost and canonical splice acceptor and donor site variants. For the missense variants we used our in-house Analysis Tool for Annotated Variants (ATAV) platform to identify the possibly and probably damaging variants based on a maximum Polyphen-2 HumDiv and HumVar prediction score[7] of greater than 0.4333. This test was applied to each autosomal gene individually as well as collectively across a set of 99 autosomal recessive neurometabolic genes published by van Karnebeek and colleagues.[8]

Second, we tested for a general effect of inherited autosomal variation by using a rare variant TDT that uses information from an independent collection of population controls (6503 EVS[9] plus 1,303 IGM sequenced controls) to weight the contribution of variants to the final test statistic.[5] In this analysis, qualifying variants were defined using the same PolyPhen-2 thresholds as above and were again required to have a global MAF less than 5%. Given that population stratification can impact the power of the test but not the type I error, we restricted this second analysis to trios with European ancestry (n=286 trios).

RESULTS

We assessed the role of inherited rare variation using the population control-weighted rare-variant TDT.[5] This test was applied to each autosomal gene across 320 eligible trios. No gene reached exome-wide significance after correcting for the 17,816 consensus coding sequence (CCDS release 14) autosomal genes (adjusted $\alpha = 2.81 \times 10^{-6}$, Table 1). Though population stratification cannot affect the false positive rate of the test, it can affect the

power.[5] We also conducted an analysis that was restricted to the 286 trios of European ancestry. Again, no gene reached the exome-wide significance level (Table 1).

We then tested for the presence of a recessive effect in each autosomal gene across the 320 trios. After quality control, only 3,472 autosomal genes were found to have at least one informative family, i.e., contain qualifying variants within the gene and that could, potentially, lead to homozygous or compound heterozygous offspring. None of these 3,472 genes achieved significance after correcting for the number of genes tested (adjusted $\alpha = 1.44 \times 10^{-5}$). The 10 most significant genes are listed in Table 2.

To investigate whether there is any evidence of recessive neurometabolic involvement in this sample, we also applied the coreTDT to the 99 autosomal recessive neurometabolic genes,[8] looking for an enrichment of homozygous or compound heterozygous offspring across the entire gene set. No enrichment was found ($p = 0.51$).

A power simulation was conducted to evaluate the types of effects that we could exclude based on this analysis. In these simulations, we conditioned on the parental genotype information contained in this IS/LGS population sample and characterized the distribution of offspring genotypes given this information and the fact that the offspring is affected. This distribution is a function of the number of causal genes, for which the family is informative, which is related to the density of causal genes within the actual gene set, and the relative risk of the offspring developing disease given that they have two affected gene copies (Supplementary Methods). Offspring are randomly sampled from this distribution and the resulting dataset is analyzed via coreTDT.[6] Since only 54 families are informative for at least one of the 99 autosomal recessive neurometabolic genes, and only 20 genes

have at least one informative family, our analyses are effectively restricted to these 54 families and 20 genes. We vary the proportion of informative genes that are actually disease causal and the relative risk and identify combinations of these parameters that attain at least 80% power (Figure 1). As can be seen, even when the compound heterozygous or homozygous qualifying variants are fully penetrant, the causal gene proportion must be larger than 40% to attain 80% power. When the proportion of causal genes is larger, e.g., 80%, we will have high power to detect an effect even with a relatively low relative risk.

Using established standards to identify pathogenic recessive genotypes[10; 11], one trio was found to have inherited two *SPATA5* pathogenic variants in a compound heterozygous manner.[12] The proband's phenotype is consistent with the *SPATA5* disease literature, and both pathogenic variants (p.Tyr559* and p.Arg84Gln) have previously been described as pathogenic among patients with *SPATA5* encephalopathy.[12]

DISCUSSION

A number of rare recessive disorders can present with an epileptic encephalopathy, particularly neurometabolic disorders; the latter are generally identified by biochemical analyses of blood, urine or CSF. We performed a global, hypothesis-free test to assess the role of autosomal recessive genetic variation in 320 patients with classic epileptic encephalopathies undiagnosed with standard clinical workups. Our sample of patient-parent trios did not identify a genome-wide significant departure in the observed number of offspring with recessive genotypes from that expected for any specific gene, or among 99 genes compiled for autosomal recessive neurometabolic disorders.

Many classical recessive metabolic disorders are routinely identified through biochemical screening prior to research study enrollment. Within our sample of 320 trios we did not find any genetic neurometabolic disorders that were missed through the conventional biochemical screening. From a clinical perspective, we emphasize that evaluation for these treatable causes should continue to be pursued. We did, however, identify a single case among the 320 with a known pathogenic recessive genotype in *SPATA5*,[12] a recently described gene for a recessive condition characterized by seizures, microcephaly, intellectual disability, and hearing loss.

The role of various dominant epilepsy genes including *ALG13*, *CDKL5*, *DNM1, GABRB3*, *SCN1A*, *SCN2A*, and *STXBP1*, for epileptic encephalopathies was securely established through exome sequencing of 356 trios and subsequent genome-wide assessments for excess *de novo* mutations identified in individual genes.[1; 2] No single gene passes a comparable threshold among the 320 trios studied here when assessing autosomal recessive genotypes. We demonstrate that the current sample of 320 trios is insufficiently powered to appropriately estimate what overall contribution autosomal recessive epilepsy genes have on the epileptic encephalopathies. Using a similar approach, a recent study on 4,125 patient-parent trios with various developmental disorders identified two novel autosomal recessive disease genes exceeding genome-wide significance,[13] emphasizing the importance of acquiring larger numbers to more confidently interpret the current lack of signal for very rare genetic epilepsies with recessive inheritance. Large-scale collaborative initiatives like the Epilepsy Genetic Initiative (EGI) and the Epi25 effort will aid the efforts to analyze genomic data on this scale.

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295 **FIGURE LEGEND**

296 **Figure 1.** coreTDT power simulation conditional on the parental genotype of 54
297 informative families and 20 informative genes in the compound heterozygous analysis.
298 Presents the combination of the relative risk and the proportion of disease causal genes
299 among these 20 informative genes, under which the tests can achieve 80% power.

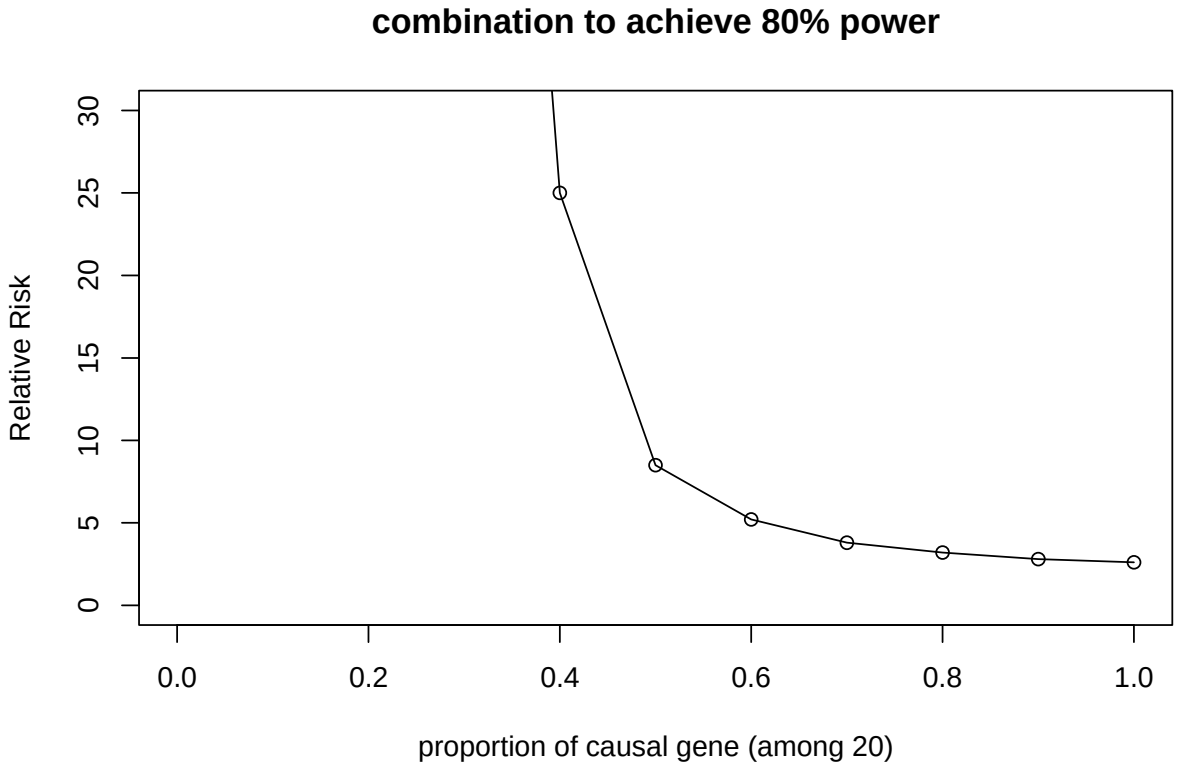

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Table 1. Top 10 genes from the analysis of rvTDT with 320 and subsequently with the subset of 286 European ancestry trios.

Test	Gene	Number of SNVs[#]	Uncorrected p-value
Analysis of rvTDT with 320 trios	*ABCA13*	52	1.17E-05
	CENPO	4	0.00236
	DST	51	0.00297
	IPPK	5	0.00198
	PCF11	10	0.00032
	SCAPER	5	0.00137
	SLC46A3	6	0.00185
	STEAP4	4	0.00291
	TRAF3IP1	7	0.00292
	ZNF878	7	0.00252
Analysis of rvTDT with 286 trios	*ABCA13*	52	0.00025
	SCAPER	5	0.00046
	ANKZF1	11	0.00079
	DST	51	0.00123
	TRAF3IP1	7	0.00172
	STEAP4	4	0.00247
	SBSN	12	0.00295
	OR2B2	5	0.00298
	SAP130	8	0.00326
	SCARF1	11	0.00344

[#]Representing the number of qualifying variants found in this gene across all families. P-value is based on the linear combination test with population controls. Adjusted α correcting for the number of genes is $p < 2.81 \times 10^{-6}$.

Table 2: Top 10 autosomal genes from the analysis of coreTDT with 320 trios

Gene	Number of SNVs[#]	Uncorrected p-value
PGM2L1	4	0.00195
CEP120	8	0.00391
CR1	25	0.00756
C14ORF177	4	0.01288
CNTRL	24	0.01563
DACT1	8	0.01563
KATNB1	10	0.01563
SYNJ1	17	0.01563
ZNF677	11	0.01563
KIAA1614	15	0.01973

[#]Representing the number of qualifying variants found in this gene across all families. Adjusted α correcting for the number of genes is $p < 2.81 \times 10^{-6}$.

Supplementary Methods

Power Simulation

Let G_f, G_m, G_o be the number of gene copies harboring a qualifying mutation in the trio's father, mother and offspring, respectively. We condition our power analysis on the observed parental genotype and study our ability to identify signal given a differing proportion of causal genes (out of the total number of genes considered), γ, and differing relative risks, R, of being diseased given two gene copies (of a causal disease gene) are affected versus less than two copies are affected. Since the analysis is conditional on the observed parental data, only a subset of genes and families are informative. [6] Specifically, only 20 genes across 54 families can have compound genotypes that lead to informative transmissions, i.e., $G_f = G_m = 1$, $G_f = 1, G_m = 2$ or $G_f = 2, G_m = 1$. 46 families are informative for only one gene and eight families are informative for two genes. In each of these eight families, the two genes are located on different chromosomes, so we assume that the transmissions of each gene are independent.

Let $D_o = 1$ indicate the fact that the offspring is affected. Let C be an indicator of whether the gene whose transmission is being considered is among the set of disease causal genes or not. When a family is informative for two genes disease causal indicators are given for each gene by C_1 and C_2. Note, we assume the disease risk for samples with multiple affected disease genes are the same with those with only one affected disease gene.

To simulate trios under the alternative, we first randomly select 20γ genes as disease causal and then generate offspring as follows.

If the family is informative for only one gene, the distribution of both offspring's gene copies being affected is given by

$$\Pr\left(G_o = 2 \middle| G_f = 1, G_m = 1, D_o = 1, C = 1\right) = \frac{R}{R+3}$$

$$\Pr\left(G_o = 2 \middle| G_f + G_m = 3, D_o = 1, C = 1\right) = \frac{R}{R+1}$$

$$\Pr\left(G_o = 2 \middle| G_f = 1, G_m = 1, D_o = 1, C = 0\right) = 0.75$$

$$\Pr\left(G_o = 2 \middle| G_f + G_m = 3, D_o = 1, C = 0\right) = 0.5.$$

If the family is informative for two genes and no more than one of them are disease causal, the compound genotype the two genes can be computed independently of one another using the equation above. When both genes are disease causal, their transmissions are not independent given the offspring is affected. In this case the compound genotypes of the offspring, for the two genes, can be given by,

$$\Pr\left(G_{o1} = 2, G_{o2} = 2 \middle| G_{f1} = G_{m1} = G_{f2} = G_{m2} = 1, D_o = 1, C_1 = C_2 = 1\right) = \frac{R}{7R+9}$$

$$\Pr\left(G_{o1} = 2, G_{o2} \neq 2 \middle| G_{f1} = G_{m1} = G_{f2} = G_{m2} = 1, D_o = 1, C_1 = C_2 = 1\right) = \frac{6R}{7R+9}$$

$$\Pr\left(G_{o1} = 2, G_{o2} = 2 \middle| G_{f1} = G_{m1} = 1, G_{f2} = 1, = G_{m2} = 2, D_o = 1, C_1 = C_2 = 1\right) = \frac{R}{5R+3}$$

$$\Pr\left(G_{o1} = 2, G_{o2} \neq 2 \middle| G_{f1} = G_{m1} = 1, G_{f2} = 1, = G_{m2} = 2, D_o = 1, C_1 = C_2 = 1\right) = \frac{R}{5R+3}$$

$$\Pr\left(G_{o1} \neq 2, G_{o2} = 2 \middle| G_{f1} = G_{m1} = 1, G_{f2} = 1, = G_{m2} = 2, D_o = 1, C_1 = C_2 = 1\right) = \frac{3R}{5R+3},$$

where G_{o1}, G_{m1}, G_{f1} and G_{o2}, G_{m2}, G_{f2} denotes the trio's compound genotypes at the first and second gene, respectively. We apply coreTDT to each simulated dataset and for each combination of γ and R, we use 1000 replicates to estimate the power. The combination of γ and R which obtains 80% power are presented in Figure 1.

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