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LETTER

Is a microRNA-328 binding site in *PAX6* associated with Rolandic epilepsy?

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Dear Editor,

We read with interest the recent publication by Panjwani et al. suggesting an association between a 3′UTR variant in the *PAX6* gene with centrotemporal spikes (CTS) and Rolandic epilepsy.[1] The authors interpret their data as implicating homozygosity of a single- nucleotide polymorphism (SNP rs662702) in the 3′UTR of *PAX6* in conferring ~12-fold increased risk of CTS and Rolandic epilepsy. This finding was based on genotyping rs662702 in a sample of 152 unrelated individuals with CTS from Rolandic epilepsy families and 1000 population controls of European origin showing homozygosity of the T allele in 6 of 152 cases (3.9% homozygosity with 95% CI of 1.5%–8.4%) and 3 of 1000 controls (0.3% homozygosity with 95% CI of 0.06%–0.9%), (odds ratio = 12.29; reported $P = 2.6 \times 10^{-4}$). There are a number of concerns surrounding this association.

Despite the earlier motivation from the authors' own linkage studies, the observed rs662702 signal remains short of conventional common variant genome-wide significance thresholds.[2,3] Also, sampling the homozygosity rate across different genetic ancestries among the Genome Aggregation Database (gnomAD; http://gnomad.broadinstitute.org/variant/11-31809070-C-T) illustrates that the homozygous carrier rate for this 3′ UTR variant is high in a number of global populations.[4] Individuals of African (8.8% homozygosity with a MAF of 30.7%), East Asian (4.1% homozygosity with a MAF of 21.5%), and Latino (3.9% homozygosity with a MAF of 22.6%) backgrounds all have equal to or greater homozygosity for the T allele than the authors' reported case homozygous rate of 3.9% (Table 1). Likewise, the 1000 Genomes Project browser (Phase 3) also describes rs662702 as having a global MAF of 17.3%, with a homozygous carrier rate of 4.5%.[5] While it could be argued that rs662702 might confer population-specific disease risk, there is very little evidence for such phenomena among the epilepsies and a more likely explanation for these discrepancies would be that rs662702 is a common population polymorphism with deviation even among residual population groups and thus the original report inadequately accounted for the confounding element of population stratification between their case and control samples.

Even for a relatively benign presentation like Rolandic epilepsy, it would be surprising to not observe some selective pressure against the T/T homozygous risk genotype. Looking among the gnomAD European control population ($n = 7281$ individuals), we find the genotype frequency for rs662702 to be 6481 (CC)/776 (CT)/24 (TT). Using the T allele frequency, a test for a departure from Hardy–Weinberg Equilibrium (HWE) in this European population sample results in an expected genotype distribution of 6480 (CC)/777 (CT)/24 (TT), (Haldane Exact test for Hardy–Weinberg equilibrium $P = 0.83$). This HWE test illustrates that there is no current evidence for selection acting against the homozygous T/T genotype within the European population, or any other population sampled in gnomAD (Table 1).

We also genotyped rs662702 among our own cohort of Rolandic epilepsy patients – a study approved by the Austin Health Human Research Ethics Committee (Project H2007/02961). Individuals were ascertained based on a diagnosis of childhood epilepsy with centrotemporal spikes (CECTS) or atypical childhood epilepsy with centrotemporal spikes (ACECTS) from Australia, Israel, and New Zealand. Of 61 eligible patients, 54 were from Australia/New Zealand (primarily European descent) and seven from Israel (primarily Middle Eastern descent). We found the T

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Table 1. Population frequency of SNP rs662702

Population	CC reference homozygotes	CT heterozygotes	TT variant homozygotes (%)	Allele (T) Frequency	HWE Exact *P*-value	Source
European (non-Finnish)	6481	776	24 (0.33%)	0.057	$P = 0.83$	gnomAD browser [1]
African	2015	1858	375 (8.83%)	0.307	$P = 0.07$	gnomAD browser [1]
European (Finnish)	1484	262	13 (0.74%)	0.082	$P = 0.64$	gnomAD browser [1]
East Asian	487	276	33 (4.15%)	0.215	$P = 0.46$	gnomAD browser [1]
Latino	224	142	15 (3.94%)	0.223	$P = 0.24$	gnomAD browser [1]
Ashkenazi Jewish	113	32	1 (0.68%)	0.116	$P = 0.69$	gnomAD browser [1]
CECTS/ACECTS European cases	50	10	1 (1.64%)	0.098	$P = 0.45$	This study

[1]Contains information from the Genome Aggregation Database (gnomAD) browser which is made available here under the Open Database License (ODbL). Data for variant 11:31809070 C/T are from 15,120 genomes (30,240 alleles) found at: http://gnomad.broadinstitute.org/variant/11-31809070-C-T.

allele homozygous in a single case (1/61, ~ 1.6% with 95% CI [0.04%–8.8%]) and heterozygous in 10 cases (10/61, ~ 16.4% with 95% CI [8.2%–28.1%]) giving an allele frequency of 9.8% (12/122 alleles) (Table 1). Two major limitations of our genotyping study are: (1) the low number of cases resulting in uncertainty over the true estimates and (2) the lack of genome-wide SNP data to more precisely estimate genetic ancestry.

The public access population genetic data raise concern about the relevance of the rs662702 homozygous T genotype in individuals ascertained for Rolandic epilepsy with CTS detected on EEG.

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Author Contributions

S.P. and M.S.H. initiated and directed the project. A.M. performed molecular genetics experiments. K.A.M., R.B., I.E.S., and S.F.B. conducted clinical phenotyping. S.P. completed statistical analysis. A.M., S.P., and M.S.H. wrote the paper. All authors discussed the results and commented on the manuscript.

Conflict of Interest

S.F.B. discloses payments from UCB Pharma, Novartis Pharmaceuticals, Sanofi-Aventis, and Jansen Cilag for lectures and educational presentations, and a patent for *SCN1A* testing held by Bionomics Inc and licensed to various diagnostic companies. I.E.S. discloses payments from UCB Pharma, GlaxoSmithKline, Eisai, Athena Diagnostics, and Transgenomics for lectures and educational presentations, and a patent for *SCN1A* testing held by Bionomics Inc and licensed to various diagnostic companies. S.P. serves on the advisory board and is an equity holder of Pairnomix. The remaining authors have no conflict of interest.

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