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Research paper

Identification of susceptibility variants to benign childhood epilepsy with centro-temporal spikes (BECTS) in Chinese Han population

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Abbreviations: BECTS, benign childhood epilepsy with centro-temporal spikes; SNPs, single nucleotide polymorphisms; GWAS, genome-wide association analysis; MAF, minor allele frequency; PCS, principal components; SMR, summary-data-based Mendelian randomization; QC, quality control; H-W, Hardy-Weinberg; EEG, electroencephalogram; ADNFLE, autosomal dominant nocturnal frontal lobe epilepsy; nAChRs, nicotinic acetylcholine receptors

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ABSTRACT

Background: Benign Childhood Epilepsy with Centro-temporal Spikes (BECTS) is the most common form of idiopathic epilepsy in children, accounting for up to 23% of pediatric epilepsy. The pathogenesis of BECTS is unknown, but it is thought that genetic factors play a role in susceptibility to the disease.
Methods: To investigate the role of common genetic variants in BECTS pathogenesis, a 2-stage genome-wide association study (GWAS) was performed in 1,800 Chinese Han BECTS patients, and 7,090 healthy controls. Genetic findings were used in a Mendelian Randomization study in the UK Biobank dataset to investigate the potential role of smoking in BECTS.
Findings: Definitive evidence of a role for common-variant heritability was demonstrated, with heritability of BECTS of >10% observed even with conservative disease prevalence assumptions. Although no individual locus achieved genome-wide significance, twelve loci achieved suggestive evidence of association ($5 \times 10^{-8} < P < 10^{-5}$). Using combined genetic and brain tissue gene expression data analyzed by Summary-data-based Mendelian Randomization (SMR), causative association of BECTS was demonstrated with SNP rs1948 and the *CHRNA5* t3603436 transcript ($P_{eqtl} = 2{\cdot}10 \times 10^{-12}$, $P_{smr} = 7{\cdot}9 \times 10^{-5}$). This finding indicates rs1948 is significantly associated with BECTS through effects on expression of *CHRNA5* in brain tissue. The identification of novel loci suggests involvements of *KALRN* and the *CHRNA5-A3-B4* cluster in BECTS. Using a generalized SMR approach we demonstrate that maternal smoking around birth is significantly associated with increased risk of BECTS (odds ratio = 3·90, $P = 0{\cdot}0099$).
Interpretation: This study shows that BECTS risk is at least partially heritable and due to common genetic variants. Additionally, we demonstrate that BECTS risk is substantially increased by maternal smoking around birth.

1. Introduction

Benign Childhood Epilepsy with Centro-temporal Spikes (BECTS) is the most common form of idiopathic epilepsy in children, accounting for 8 to 23% of epilepsy in children less than 16 years of age [1,2]. The typical age of onset is 3 to 14 years, and the condition typically resolves by the early teenage years. Whilst affected children are usually neurodevelopmentally normal, BECTS has been associated with varying degrees of neuropsychological damage, and can be associated with sociological and behavioral problems in adulthood [3].

Increased familiality of BECTS has raised the hypothesis that the condition has genetic underpinnings (reviewed in [4]). However familial recurrence may occur because of shared environmental or genetic factors, and to date formal heritability studies in twins have not supported a major genetic contribution to susceptibility [5]. To better characterize the genetic architecture of BECTS, a large two-stage case-control GWAS was conducted in the Han Chinese population of 1800 BECTS cases and 7090 healthy ethnically matched controls. A significant common variant heritability of the disease was demonstrated, and several loci identified with suggestive association with BECTS risk.

Research in context

Evidence before this study

We investigated all the manuscripts on BECTS in PubMed and China National Knowledge Infrastructure (CNKI in Chinese language). BECTS is the most common form of idiopathic epilepsy in children. Whilst increased familiality of BECTS has raised the hypothesis of BECTS to be a genetic disorder, it remains elusive whether there is a major genetic contribution to susceptibility. To date, formal heritability studies in twins have not supported the hypothesis, and it has not been investigated in a GWAS study to pinpoint the genetic predisposition and further the disease causality.

Added value of this study

Here, we identified 12 loci suggestively associated with BECTS loci in a case-control GWAS study of Chinese Han population. The post-GWAS analysis indicates BECTS associated variant rs1948 is significantly associated with BECTS through effects on expression of CHRNA5 in brain tissue. Using a generalized SMR approach we demonstrate that maternal smoking around birth is significantly associated with increased risk of BECTS. Further, significant common variant heritability of BECTS was observed in the combined phase 1 and 2 datasets which was > 10% even for assumed prevalence as low as 0.00025.

Implications of all the available evidence

It suggests the involvements of multiple susceptible genes in pathogenesis of BECTS. BECTS risk is at least partially heritable and explained by common genetic variants. Additionally, maternal smoking around birth is casually associated with onset of BECTS in offspring.

2. Materials and methods

2.1. Subjects

Patients with BECTS were recruited from outpatient clinics at 30 hospitals in Beijing, Shanghai, and 26 other Chinese provincial capital cities. Diagnosis was determined according to the International League Against Epilepsy (Commission on Classification and Terminology of the International League Against Epilepsy, 1989)[6] definition for BECTS by pediatric neurologists. A patient was diagnosed with epilepsy if he/she had at least two unprovoked epileptic seizures. Healthy, unrelated adult blood donors, from the Beijing and Shanghai were included as controls. Only self-reported Han Chinese ethnicity cases and controls were recruited, and additional ethnicity checks were performed as described below. Written informed consent was obtained from all the parents or guardians, or directly from adult participants, and the study was approved by the relevant ethics committees of the hospitals and institutions involved.

2.2. Genotyping and quality control

DNA was isolated from venous blood samples of study participants. Genome-wide genotyping was performed with Illumina OmniZhongHua-8 version1.0 BeadChips (Stage 1) and Illumina

Human CoreExome-24 version 1.0 BeadChips (Stage 2) on an Illumina iScan array scanner at the Laboratory of Department of Rheumatology and Immunology, Changzheng Hospital (Shanghai, China). Genotype calls were made using the Illumina BeadStudio software; all SNPs with quality scores <0·15 were excluded. The cluster plots of the top-associated single nucleotide polymorphisms (SNPs) were inspected manually.

Genome-wide association analysis was performed using PLINK. We excluded individuals with call rates below 98% and heterozygosity rates >3 standard deviations from the mean. Duplicate subjects or probable relatives were identified by identify-by-descent (IBD) analysis (PI_HAT>0·1875) and excluded.

SNP markers were excluded if they had a minor allele frequency (MAF) below 0·01, a genotype distribution out of Hardy-Weinberg equilibrium ($P<10^{-7}$), or had a high rate of missing genotype calls (missing genotype call rate>0·02). Outliers on heterozygosity vs missingness plots were also excluded (Supplementary Figure 1). Sex chromosomes were excluded from the analysis; only autosomal SNPs were analyzed. After these quality control steps, to detect and correct for population stratification we used the Shellfish software (http://www.stats.ox.ac.uk/~davison/software/shellfish/shellfish.php), having first excluded regions of long range LD. To confirm ethnicity, we performed a continental PCA on the Han Chinese dataset, merged with available data from 51 available populations genotyped by Illumina 650Y from the Human Genome Diversity Panel (HGDP-CEPH) [7]. Continental PCA indicated that all the samples came from subjects of Han Chinese descent (East Asian) (Supplementary Figure 2). Cases or controls lying more than 6 standard deviations from the population mean on principal components (PCs) 1−10 were then excluded (Supplementary Figure 3).

2.3. Data analysis

Imputation was performed separately according to SNP microarray used, using the 1000 Genome reference through the Sanger Imputation Service (imputation.sanger.ac.uk/). BECTS associations of all markers with a MAF>0.01 and imputation quality INFO>0·8 (6,563,936 SNPs in the OmniZhonghua set and 5,742,369 SNPs in the CoreExome set) were analyzed by using logistic regression (PLINK) with dosage output, adding the top PCs as covariates (4 PCs for the OmniZhonghua set; 3 PCs for the CoreExome set). The selection of the numbers of PCs that used to control for population stratification was based on Scree plot findings (Supplementary Figure 4) and calculation of lambda was performed after controlling for top PCs. A meta-analysis was then performed combining SNPs genotyped or imputed in both datasets using METAL software [8].

Heritability was calculated by GCTA software [9] using both observed and liability scales, the latter for population prevalences of 0·00025, 0·0005, 0·001, 0·002, 0·003, 0·004, and 0·005. Because small errors for each SNP can accumulate to give incorrect estimates for genetic variance, for this analysis additional, extremely stringent, QC steps were employed. We excluded SNPs whose p values were <0·05 for either the Hardy-Weinberg equilibrium test or for missingness-difference between cases and controls. To keep individuals who were only distantly related, both individuals from a pair with an estimated relationship statistic PI_HAT>0·05 were excluded.

The Summary-data-based Mendelian Randomization (SMR) analysis method [10] was used to further analysis the suggestive GWAS hits obtained in our study. This approach analyses the intersection of genetic effects on gene-expression (eQTLs) and on the trait/disease, to determine the most likely candidate gene at individual loci. We used SMR analysis to prioritize these candidate genes on the basic of the GWAS and eQTL data (BRAINEAC eQTL data from brain tissue samples. http://www.braineac.org/).

A generalised SMR (GSMR) analysis was performed to investigate the relationship between smoking and risk BECTS [11]. Summary-level GWAS data from the current study and from analysis of "maternal smoking around birth" from the UK Biobank (Data-Field 1787, $N_{case/control}$ = 95,182/214,760; total N = 309,942, http://www.nealelab.is/uk-biobank/) were analysed, using the Complex Traits Genetics Virtual Lab platform (https://vl.genoma.io)[12]. Outlier SNPs that have apparent pleiotropic effects on both maternal smoking around birth and BECTS and could therefore bias the GSMR findings were identified and excluded using the heterogeneity in dependent instruments (HEIDI)-outlier method.

3. Results

3.1. Genome-wide association analysis

The first stage of GWAS included individuals of 997 BECTS cases and 3115 healthy controls. 972 cases and 2916 controls genotyped using the Illumina OmiZhongua SNP microarray for 805,150 SNPs were retained after a series of stringent quality control (QC) procedures. Minimal residual genomic inflation was observed (genomic inflation factor after correcting top 4 PCs=1·033) (Supplementary Figure 5A). The second stage GWAS was performed with an independent cohort of 803 BECTS cases and 3975 controls. 777 cases and 3768 controls were genotyped using the Illumina Core-Exome SNP microarray for 257,007 SNPs and left/remained for association analysis after QC procedures. Again, minimal residual genomic inflation was observed (genomic inflation factor=1·034) (Supplementary Figure 5B) after correcting for the top 3 principal components (PC). SNP imputation was performed on each stage (imputed lambda of OmniZhonghua and CoreExome cohorts are 1·042 and 1·039 respectively) and imputed genotype data was combined by meta-analysis (final analysis 5,352,724 SNPs in 1738 cases and 6592 controls). Whilst no locus in this analysis achieved genome-wide significance ($P<5\times10^{-8}$), 12 independent loci reached suggestive significance ($5\times10^{-8}<P<10^{-5}$, Table 1, Supplementary Figure 6), the direction of association being consistent between datasets for each locus ($P = 2{\cdot}44\times10^{-4}$).

The strongest associations observed were multiple SNPs in regions on chromosome 3, 15 and 10, located in or nearby the genes *KALRN*, *CHRNB4*, and *PTCHD3*/*RAB18* (zoom plots, see Figs. 1A-B and Supplementary Figure 6A, respectively). The most strongly associated SNP, rs1561578 (Fig. 1A), is within an intron of *KALRN*, which encodes the protein kalirin. The second associated signal rs1948 was encompassed by several genes that encode nicotinic cholinergic receptor subunits (*CHRNB4*/*CHRNA5*/*CHRNA3*ç Fig. 1B). The third locus on chromosome 10p12.1 (peak SNP rs139905806, Supplementary Figure 6A) is a region which has been previously associated with lamotrigine-induced skin rash in a previous GWAS study in Korean patients with epilepsy [13].

3.2. Summary-data-based mendelian randomization analysis

eQTL analysis of the probes of the 12 most strongly-associated loci demonstrated that 9 were associated with transcriptional levels of the most proximal gene ($P_{eqtl}<0{\cdot}05$). To investigate whether the observed genetic associations operated through these transcriptional effects, a Summary-data-based Mendelian Randomization (SMR) analysis was performed, using brain tissue gene-expression data. Significant association was observed at *CHRNA5* locus, tagged by rs76712448 (P_{smr}=0·028, Fig. 2). A HEIDI test supported this SNP being directly associated with BECTS and CHRNA5 expression. The most strongly associated GWAS hit, rs1948, shows significant association at the *CHRNA5* loci in the SMR analysis ($P_{smr}=7{\cdot}9\times10^{-5}$). The association suggests that more than one SNP on the haplotype tagged by rs1948 is associated with BECTS susceptibility through effects on central nervous system (CNS) *CHRNA5* expression.

Table 1
SNPs achieving suggestive association ($10^{-5} < P < 5 \times 10^{-8}$) in the meta-analysis of imputed GWAS data. Findings are given for the most strongly associated SNP at each locus.

SNP	Chr	BP	Gene	Left gene	Right gene	Allele 1/ Allele 2	Frequency Allele 1	Odds Ratio	95% CI	P-meta	Direction	Heterogeneity P value
rs73141536	3	85,653,011	*CADM2*	*NA*	*NA*	T/G	0.19	1.26	1.13–1.39	5.11×10^{-6}	++	0.075
rs1561578	3	124,328,506	*KALRN*	*ROPN1*	*UMPS*	A/C	0.11	0.71	0.60–0.82	1.58×10^{-6}	−−	0.80
rs9814627	3	141,920,434	*GK5*	*TFDP2*	*XRN1*	A/G	0.51	0.83	0.76–0.90	4.78×10^{-6}	−−	0.95
rs34397315	4	133,753,668	NA	*LOC646187*	*PCDH10*	T/C	0.46	0.83	0.76–0.90	8.29×10^{-6}	−−	0.019*
rs10519952	4	149,201,414	*NR3C2*	*ARHGAP10*	*ASS8*	A/G	0.80	1.27	1.13–1.41	8.54×10^{-6}	++	0.69
rs139905806	10	27,710,533	NA	*PTCHD3*	*RAB18*	A/G	0.72	0.81	0.73–0.88	1.98×10^{-6}	−−	0.20
rs2175709	12	64,107,953	NA	*DPY19L2*	*TMEM5*	A/T	0.35	0.82	0.74–0.89	8.29×10^{-6}	−−	0.89
rs12230762	12	105,490,149	NA	*ALDH1L2*	*KIAA1033*	C/G	0.75	1.24	1.12–1.37	9.23×10^{-6}	++	0.87
rs9317149	13	61,477,831	NA	*LOC390407*	*PCDH20*	T/C	0.017	1.91	1.30–2.51	5.03×10^{-6}	++	0.57
rs28405640	14	104,775,852	NA	*C14orf144*	*LOC100131034*	C/G	0.62	1.23	1.12–1.34	2.07×10^{-6}	++	0.58
rs1948	15	78,917,399	*CHRNB4*	*CHRNA3*	*LOC390612*	A/G	0.47	1.22	1.12–1.32	1.83×10^{-6}	++	0.089
rs60419110	18	7,880,252	*PTPRM*	*LRRC30*	*LOC100128219*	T/C	0.54	1.21	1.11–1.31	4.39×10^{-6}	++	0.18

Chr = chromosome, BP = base pair position, hg18. Gene = gene closest to strongest associated SNP. Left, Right gene = left- and right-flanking genes, most nearby coding genes from the strongest associated SNP. P-meta = *p*-value from meta-analysis. Direction of association = direction of odds ratio in the individual dataset.
* Heterogeneity analysis showed that the associations of this SNP in each sets were significant different (Heterogeneity P value < 0.05). The separate LocusZoom plots in each datasets of this locus were attached in the supplementary files (Supplementary Figure 7).

3.3. Generalised summary-data-based mendelian randomisation analysis

To test for potential causal association between maternal smoking around birth and BECTS, we conducted a GSMR analysis. GSMR algorithm selected 5 independent SNPs as instruments from the GWAS of maternal smoking at birth at a genome-wide significance level ($P_{GWAS} < 5 \times 10^{-8}$, Supplementary Table 3). We identified a risk effect of maternal smoking around birth for BECTS (b_{xy}=1.36 for BECTS is approximately equivalent to odds ratio = 3·90, P = 0·0099) (Fig. 3).

3.4. Heritability of BECTS

Common variant heritability was tested using the unrelated cases and controls from the combined imputed dataset. The h^2 estimates were calculated for a range of BECTS prevalences (Fig. 4, Supplementary Table 2), showing that h^2 captured by these SNPs was > 0·10 for prevalence 0·00,025.

4. Discussion

BECTS is the prototypic illness of a group of epileptiform diseases characterized by the electroencephalogram (EEG) finding of rolandic spike and wave discharges (predominantly centro-temporal spikes). Despite findings from twin studies not supporting a genetic etiology, the tendency of BECTS to run in families has spurred efforts to identify genetic variants associated with the disease including candidate gene association studies, and sequencing studies aimed at identifying rare variant associations. To date though no robust genetic association has been reported with the condition.

This study demonstrates that BECTS does have significant common variant heritability. The cumulative incidence studies indicate that up to the age of 15 years, 1·0–1·7% of children will have at least one unprovoked seizure, and 0·7–0·8% repeated seizures [14], and BECTS represents ~15% of childhood epilepsy [15]. This study demonstrates that even assuming a prevalence or a lifetime risk of BECTS as low as 2·5/10,000, the common variant heritability of the condition remains > 0·10. Assuming a disease prevalence of 0·1%, the liability scale heritability in the combined phase 1 and 2 datasets was 0·123 (standard error 0·020, $P = 3{\cdot}74 \times 10^{-11}$), reflecting the heritability captured by the SNPs genotyped on the two microarrays used and imputed from them. While conducting GWAS analysis, rare SNPs with MAF<1% were excluded, and thus this heritability figure does not include rare variants effects. Nor does the association analyses include heritability of other forms of genetic variation that may be either missed or incompletely captured by the SNPs studied, such as copy number variation, chromosomal rearrangements or epigenetic changes. Unlike family or twin studies which make assumptions regarding sharing of environmental risk factors, the GCTA approach employed here makes no such assumption, confirming that the risk of this trait is significantly determined by common genetic variants. The absence of major gene effects in the GWAS conducted in this study indicates that the disease is likely to be largely polygenic in pathogenesis. These findings are consistent with common variant heritability reported for epilepsy overall (26%, standard deviation 5%), for focal seizure epilepsy (27%, standard deviation 5%)[16], or for epilepsy subtypes (4·1–107%)[17], though neither of these studies specifically included BECTS patients.

Suggestive association of BECTS was observed with 12 SNPs with concordant association in both datasets. These associations are novel, and no association was observed with genes previously implicated in BECTS and related forms of epilepsy, including *ELP4* [18], *GRIN2A* [19-21] and *RBFOX1*/*RBFOX3*[22,23]. Because the SNP microarrays used in the current study do not detect the rare variants previously reported in these genes, this study simply shows that common variants in

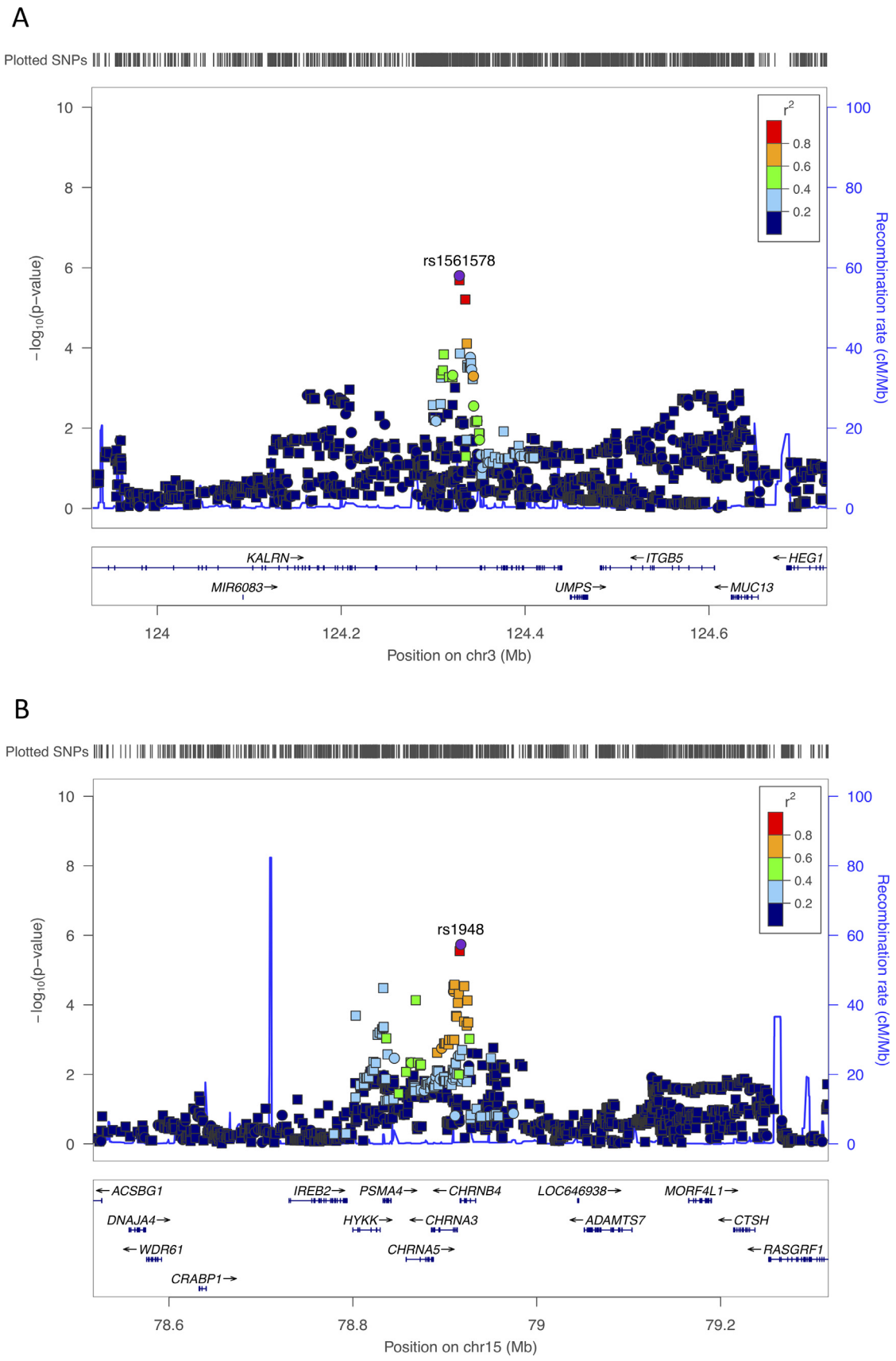

Fig. 1. A) The top SNP, rs1561578, is shown in purple, and the remaining SNPs are colored according to their linkage disequilibrium r2 value with rs1561578. Genotyped SNPs in OmniZhonghua set are shown as dots and imputed SNPs shown as squares. B) The top SNP, rs1948, is shown in purple, and the remaining SNPs are colored according to their linkage disequilibrium r2 value with rs1948. Genotyped SNPs in OmniZhonghua set are shown as dots and imputed SNPs shown as squares.

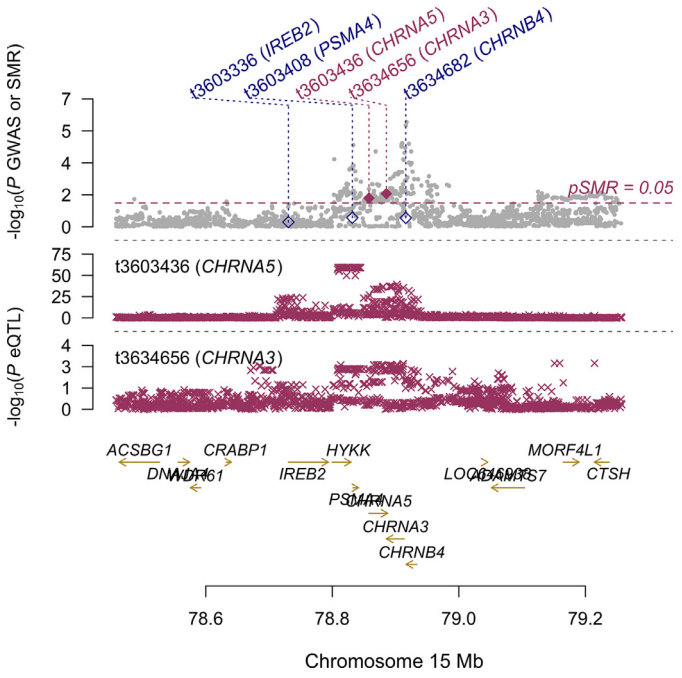

Fig. 2. Prioritizing genes at a GWAS locus using SMR analysis. The findings for chromosome 15p24 locus for BECTS are displayed. In the top plot, gray dots represent the P values for SNPs from the GWAS meta-analysis for BECTS, and diamonds represent the P values for probes from the SMR test. The middle plot presents the eQTL P values of SNPs from the BRAINEAC study for the transcripts tagging CHRNA5 and CHRNA3. The top and middle plots include all the SNPs available in the region in the GWAS and eQTL summary data, respectively, rather than only the SNPs common to both data sets.

these genes (MAF>1%) do not have major influences on the risk of BECTS.

A combination of suggestive genetic association and strong transcriptomic effect supports the involvement of the gene *CHRNA5*, encoding the cholinergic receptor nicotinic alpha 5 subunit, in BECTS aetiopathogenesis. While the most strongly associated SNP at this locus (rs1948 at chromosome 15q24) is located within the gene *CHRNB4*, SMR analysis suggests that the associated gene at this locus is the neighbouring gene *CHRNA5*. The rs1948 is strongly associated with expression of the t3603436 transcript of the *CHRNA5* genes (P_{eqtl}=2·10 × 10^{-12}, P_{smr}=7·9 × 10^{-5}) which encodes a subunit of cholinergic receptors. Acetylcholine is an important excitatory CNS neurotransmitter. Association has previously been reported between multiple SNPs in the *CHRNA5-CHRNA3-CHRNB4* gene cluster and cigarette smoking, nicotine dependence, and smoking associated lung diseases [24-26], as well as with cognitive measures [27,28]. There is suggestive evidence that smoking increases the risk of epilepsy overall [29], but whether smoking influences the risk of BECTS specifically is unknown. It was reported that BECTS risk allele rs1948-A is also associated with higher Fagerström Test for Nicotine Dependence (FTND) score in the Chinese Han population [30,31]. Although it is unlikely the patients themselves were smoking at their age of onset, they could have been exposed to secondhand smoke. If the offspring is a carrier of the risk allele, then at least one of the parents must be a carrier too, which means they are more likely to develop tobacco addiction or become a heavier smoker. Studies have shown that prenatal maternal cigarette smoking is associated with febrile seizure [32,33]. It is possible that *CHRNA5* influences the onset of BECTS by another mechanism independently of smoking (including maternal or paternal perinatal, or postnatal passive, smoking). The GSMR

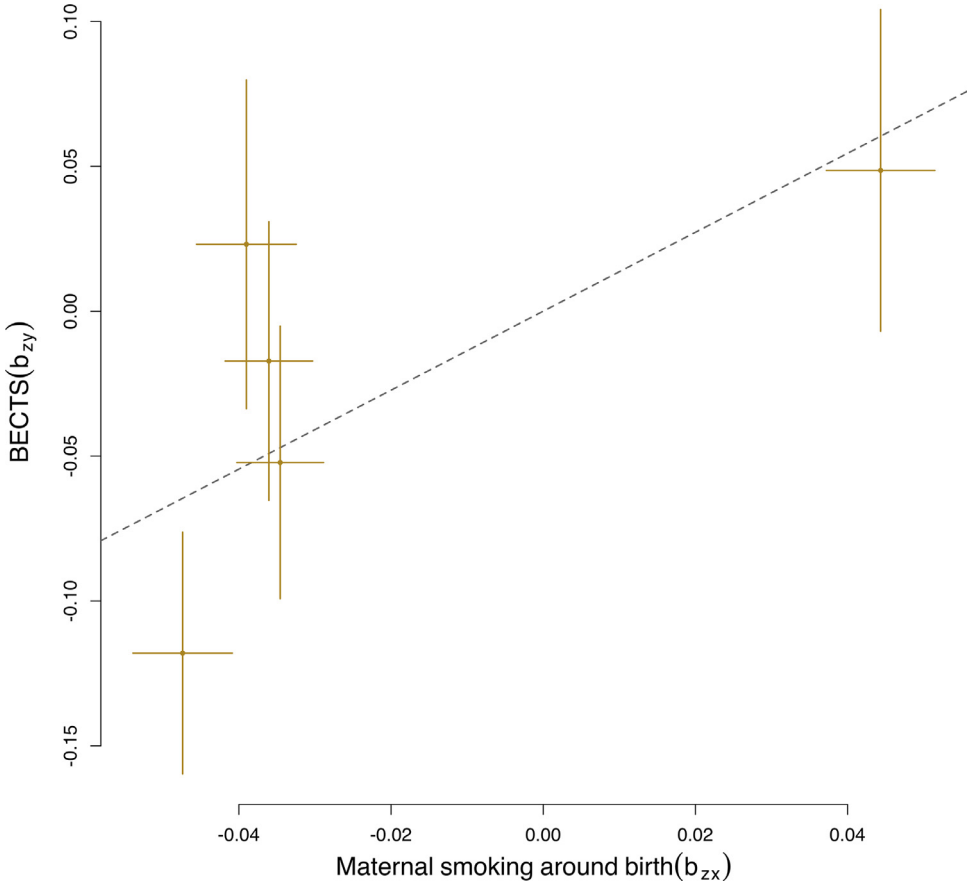

Fig. 3. GSMR analysis to test for the effect of maternal smoking around birth on BECTS. The plot shows the relationship between the estimated effects of SNPs (z) associated with maternal smoking around birth (x) on BECTS (y axis, bzy) and the estimated effect of z on x (x axis, bzx). The slope of the dashed line represent the ratio between the bzy and bzx as the estimate of the mediation effect of x on y (bxy = bzy / bzx). Error bars in represent the standard errors.

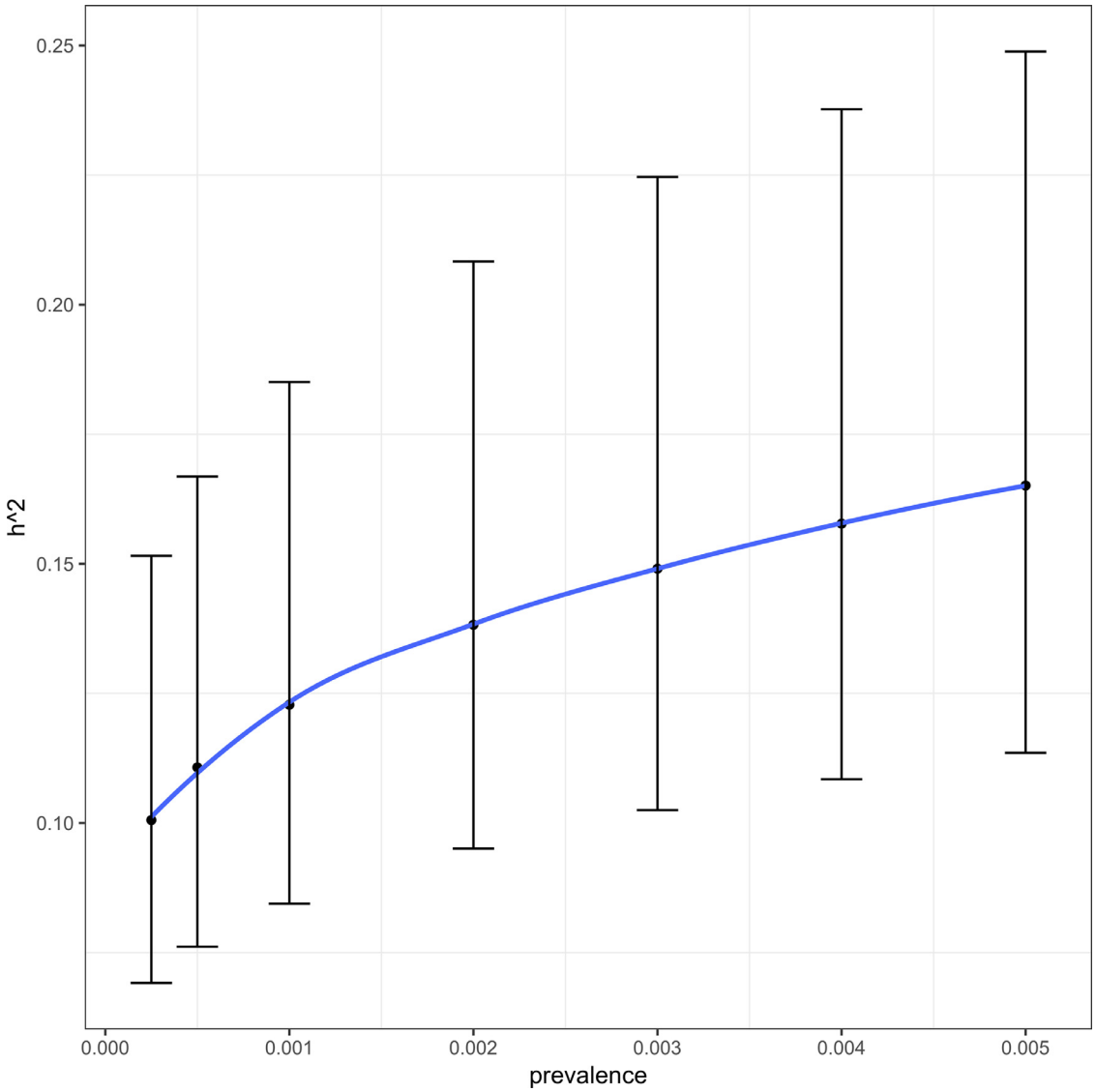

Fig. 4. Estimated common-variant SNP heritability of BECTS (y axis, h2) in relation to disease prevalence (x axis,%), with 95% confidence intervals indicated by vertical bars. GCTA heritability analysis calculates using the liability scale. The estimate of variance explained on the observed scale is transformed to that on the liability scale, Proportion of cases in the sample = 0.21; User-specified disease prevalence = 0.00025, 0.0005, 0.001, 0.002, 0.003, 0.004, 0.005).

analysis reported here though demonstrates using data from the UK Biobank that maternal smoking around birth is associated with 3·9x increased risk of BECTS. Unfortunately data is not available in UK Biobank about other forms of perinatal or antenatal smoking exposure, and therefore we are unable for example to determine the effect of paternal or other sources of passive smoking on BECTS risk.

Linkage of chromosome 15 (15q24), at which *CHRNA3, CHRNA5*, and *CHRNB4* are encoded, has also been reported in autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) [34], but this finding has not been confirmed in other families [35,36], and the mutations identified to date in *CHRN* genes in ADNFLE have been in other receptor subunits (reviewed in [37]). Of interest, as with ADNFLE, in BECT seizures occur more commonly at night and during sleep, suggesting overlapping pathogenesis. Studies in rodents have described the anatomical localization and function of the nicotinic acetylcholine receptors (nAChRs) formed by the subunits encoded by this gene cluster. Animal experiments also have shown that microinjection of acetylcholine into the brain can cause seizures in animals, suggesting that acetylcholine, as a neurotransmitter, may play an important role in the development of epilepsy [38,39]. While not definitive, this data supports a role for this locus and a possible link between nicotine/cholinergic neurostimulation and BECTS, raising the hypothesis that anticholinergic therapies may be effective in BECTS. Further research will be required to test this.

KALRN, associated with Heschl's gyrus (temporal lobe) morphology in GWAS, induces various signaling mechanisms that regulate neuronal shape, growth, and plasticity, through their effects on the actin cytoskeleton [40]. This locus has not previously been associated with epilepsy but its known association with brain morphology is consistent with a role in a CNS disease like this. Another eight loci achieved suggestive association, and further research will be required such as expanding the GWAS dataset, and/or functional genomic analyses, to determine if they have a role in BECTS.

In conclusion, this study shows that significant common variant heritability contributes to the development of BECTS, and provides evidence that the cholinergic receptor subunit gene *CHRNA5*, is involved in its pathogenesis. This raises the hypothesis that anticholinergic therapies may be effective in BECTS; further research will be required to test this. Importantly, the study demonstrates through Mendelian randomization approaches that maternal smoking around birth is associated with increased risk of BECTS, thereby identifying to our knowledge the first environmental risk factor for the disease.

Author contributions

Huji Xu, Li-Ping Zou, Perry Bartlett, David Reutens and Matthew A Brown made substantial contributions to conception and design of the study. Geng Wang, Zhixiu Li, Paul Leo, Gabriel Cuellar Partida, Mischa Lundberg and Matthew A Brown contributed to statistical analysis and interpretation of data. Huji Xu, Li-Ping Zou, Xiuyu Shi, Zhisheng Liu, Gefei Wu, Hongmin Zhu, Yuqin Zhang, Dong Li, Li Gao, Liu Yang, Wei Wang, Jianxiang Liao, Jiwen Wang, Shuizhen Zhou, Hua Wang, Xiaojing Li, Jingyun Gao, Li Zhang, Xiaomei Shu, Dan Li, Yan Li, Chunhong Chen, and Xiuju Zhang were responsible for case diagnosis, subject recruitment and the collection of blood samples. David Reutens are responsible for reviewing the diagnosis of the patients. Geng Wang, Matthew Brown, and Huji Xu were primarily responsible for drafting the manuscript and revising it. All authors approved the final version of the manuscript.

Declaration of Competing Interest

The authors have no conflict of interest.

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Supplementary materials

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