Tye Charlotte (Orcid ID: 0000-0002-8567-9547)
McEwen Fiona (Orcid ID: 0000-0002-5562-481X)
Sheerin Fintan Brian John (Orcid ID: 0000-0002-9891-7612)

Epilepsy severity mediates association between mutation type and ADHD symptoms in tuberous sclerosis complex

Charlotte Tye, Department of Child & Adolescent Psychiatry, Institute of Psychiatry, Psychology & Neuroscience, King's College London. Email: charlotte.tye@kcl.ac.uk ORCID: 0000-0002-8567-9547[1]

Fiona S. McEwen, Department of Child & Adolescent Psychiatry, Institute of Psychiatry, Psychology & Neuroscience, King's College London. Email: f.mcewen@qmul.ac.uk [1,2]

Holan Liang, Department of Child & Adolescent Psychiatry, Institute of Psychiatry, Psychology & Neuroscience, King's College London. Email: holan.liang@gmail.com[3]

Emma Woodhouse, Department of Child & Adolescent Psychiatry, Institute of Psychiatry, Psychology & Neuroscience, King's College London. Email: emma.woodhouse@kcl.ac.uk [4,5]

Lisa Underwood, Department of Child & Adolescent Psychiatry, Institute of Psychiatry, Psychology & Neuroscience, King's College London. Email: l.underwood@auckland.ac.nz[6]

Elizabeth Shephard, Department of Child & Adolescent Psychiatry, Institute of Psychiatry, Psychology & Neuroscience, King's College London. Email: lizzieshephard@gmail.com[7]

Edward D. Barker, Department of Psychology, Institute of Psychiatry, Psychology & Neuroscience, King's College London. Email: ted.barker@kcl.ac.uk

Fintan Sheerin, Department of Neuroradiology, Oxford University Hospital NHS Foundation Trust. Email: fintansheerin@gmail.com

Nicholas Higgins, Department of Radiology, Addenbrooke's Hospital, Cambridge University Hospitals NHS Trust. Email: nick.higgins@addenbrookes.nhs.uk

Juul Steenbruggen, Department of Child & Adolescent Psychiatry, Institute of Psychiatry, Psychology & Neuroscience, King's College London. Email: juul_29@hotmail.com

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/epi.17507

Patrick F. Bolton, Department of Child & Adolescent Psychiatry, Institute of Psychiatry, Psychology & Neuroscience, King's College London. Email: patrick.bolton@kcl.ac.uk ORCID: 0000-0002-5270-6262[10]

Current affiliations:

1. Department of Psychology, Institute of Psychiatry, Psychology & Neuroscience, King's College London
2. Department of Biological and Experimental Psychology, School of Biological and Behavioural Sciences, Queen Mary University of London
3. Great Ormond Street Hospital NHS Trust, London.
4. Forensic and Neurodevelopmental Sciences, Institute of Psychiatry, Psychology & Neuroscience, King's College London
5. Compass Psychology Services, Bromley
6. Department of Population Health, University of Auckland
7. Department of Psychiatry, University of São Paulo, Brazil
8. Department of Neuroradiology, Oxford University Hospital NHS Foundation Trust
9. Department of Radiology, Addenbrooke's Hospital, Cambridge University Hospitals NHS Trust
10. Social, Genetic & Developmental Psychiatry Centre, Institute of Psychiatry, Psychology & Neuroscience, King's College London

SUMMARY

The association between attention deficit hyperactivity disorder (ADHD) and tuberous sclerosis complex (TSC) is widely reported, with support for the role of epilepsy, yet the mechanisms underlying the association across development are unclear. The Tuberous Sclerosis 2000 Study is a prospective longitudinal study of TSC. In Phase 1 of the study, baseline measures of epilepsy, cortical tuber load and mutation were obtained with 125 children aged 0 – 16 years. In Phase 2, at an average of 8 years later, ADHD symptoms were measured for 81 of the participants. Structural equation modelling revealed an indirect pathway from genetic mutation, to cortical tuber load, to epileptic spasm severity in infancy, to ADHD symptoms in middle childhood and adolescence, in addition to a pathway linking current seizure severity to ADHD symptoms. Findings were retained when IQ was entered as a correlated factor. The findings support a cascading developmental pathway to ADHD symptoms mediated by early-onset and severe epilepsy in the first two years of life. This warrants detailed investigation of seizure characteristics and cognitive and behavioural sequelae associated with ADHD from early in life, to further understanding of the association between ADHD and early-onset epilepsy across syndromic and non-syndromic populations.

Keywords: ADHD, epilepsy, longitudinal, tuberous sclerosis complex

INTRODUCTION

Tuberous sclerosis complex (TSC) is a genetic condition caused by mutations in the TSC1 and TSC2 genes, which lead to marked upregulation of the mammalian target of rapamycin (mTOR) pathway and consequently the development of hamartamous lesions[1]. Cortical tubers and subependymal

nodules are associated with a high prevalence of epilepsy in TSC, which occurs in up to 90% of individuals and often begins in the first year of life[2]. In addition to the physical manifestations, individuals with TSC may also experience an array of intellectual, neurodevelopmental, behavioural, psychiatric and psychosocial difficulties, recently termed TSC-associated neuropsychiatric disorders (TAND[3]). Diagnosis and symptoms of attention deficit hyperactivity disorder (ADHD) are prevalent in TSC, with wide-ranging estimates of prevalence from 20% to 60%[4; 5]. Individuals with TSC and ADHD diagnosis or symptoms are more likely to have a low IQ and/or diagnosis of intellectual disability (ID), and co-occurring diagnoses, including autism and mood or anxiety disorders[5].

While the association between TSC and ADHD is widely reported, little is known about the possible neurobiological mechanisms underlying it[6]. Although TSC1/TSC2 mutations appear to have a similar frequency in individuals with an ADHD diagnosis[3], downstream effects of the mutation on neural development may be implicated. Cortical tubers are likely to impact brain development, for example frontal systems involved in regulatory activities associated with ADHD, which have been associated with cognitive ability in TSC[7]. There is substantial support for the role of epilepsy in ADHD, regardless of cause[8], and a history of seizures, intractable epilepsy and epileptic spasms have been associated with ADHD symptoms in TSC[4; 9]. While the limited research to date suggests that the association between TSC and ADHD is likely to be multifactorial in nature, the majority of studies have relied on retrospective report and chart review. In a well-characterised prospective longitudinal cohort study, we aimed to chart multifactorial longitudinal pathways to individual differences in ADHD symptoms using structural equation modelling, with a focus on the role of epilepsy.

METHODS

See also Supplementary Material Section 1.

Participants

The TS 2000 Study is a population-based, prospective longitudinal study of the natural history of TSC[10]. In Phase 1 of the study (2001-2005), 125 participants met diagnostic criteria for TSC and completed study assessments (n=62 male, median age=39 months, range=4-254). At the initial assessment, a full medical history was obtained and a physical examination carried out using a standardized protocol. Full details of the study assessment protocol have been reported elsewhere[10]. During Phase 2 of the study (2012-2015), clinical researchers gathered information on behaviour and development, including ADHD symptoms with 74 of the participants (male n=33, median age=147 months, range=93-323) and intellectual abilities with 88 of the participants (male n=39, median age=148 months, range=93-323), at an average of 8.3 years (range 5.5-10.8) after Phase 1. A medical ethics committee approved the study protocol (Research Ethics Committee ref: 00/7/061). Written informed consent was obtained prior to assessment.

Measures

Clinical features of TSC

A causal TSC mutation was determined for 96 children (TSC1 n=19; TSC2 n=77). Copies of clinical brain scans were obtained from the hospitals where imaging had been conducted during Phase 1 and 2 (n=109) and coded for number and location of cortical tubers and subependymal nodules. A tuber total latent factor score was calculated for each participant using confirmatory factor analysis[7]. Seizure severity scores were generated using the E-Chess[11]. A factor score was calculated for each participant for the first and second years of life (n=120) and the 3-month period leading up to the Phase 2 assessment (current seizure severity; n=94), comprising number of seizure types, time period over which seizures occur, seizure frequency at most severe, number of anti-epileptic drugs used and response to treatment. To disentangle the effect of epileptic spasms from other seizure types

(including generalised and focal seizures) on ADHD symptoms, spasm severity was separately coded from "non-spasm seizure severity" for the first and second year of life, when spasms tend to occur.

ADHD symptoms

A primary caregiver completed the Strengths and Difficulties Questionnaire (SDQ; n=74) and the Development and Wellbeing Assessment (DAWBA; n=54). The 'hyperactivity' subscale of the SDQ was used to characterize ADHD symptoms, and diagnostic symptom counts were calculated based on parent ratings of DSM-5 ADHD criteria on the DAWBA, separately for inattention and hyperactivity/impulsivity (maximum 9 each) symptoms. These measures were used in combination with diagnostic interviews, the Schedule for Affective Disorders and Schizophrenia for School-Age Children or Diagnostic Interview for ADHD in Adults, to define a multi-informant best-estimate clinical diagnosis (n=81; authors PB, HL).

Intellectual ability

Estimated IQ was available for 88 participants, combining scores on the Wechsler Abbreviated Scale of Intelligence – Second Edition (WASI-2; n=57), British Picture Vocabulary Scale (BPVS; n=1) and the Vineland Adaptive Behaviour Scales, Second Edition (VABS-II; n=81). The adaptive behaviour composite from the VABS-II was used to estimate IQ when the participant was not able to complete the WASI-2 or BPVS, either due to level of functioning (n=24) or when administration of the WASI-2 was not possible (n=2[7]).

Statistical analysis

Structural equation modelling was conducted in MPlus (version 7.31) to test indirect pathways from genetic mutation to ADHD symptoms measured by the SDQ and DAWBA symptom counts for inattention and hyperactivity/impulsivity (see Figures 1 and 2). To account for missing values, full information maximum likelihood estimation was used. A bootstrap model (1000 resamples) was used to estimate the standard errors of parameter estimates and the bias-corrected confidence intervals of the indirect effects.

RESULTS

Prevalence of ADHD symptoms in TSC

A significant proportion of participants demonstrated elevated ADHD symptoms on the SDQ (17.5% (n=13) borderline ADHD symptoms; 36.5% (n=27) high ADHD symptoms) and DAWBA (37% (n=20) met DSM-5 criteria for inattention, 11% (n=6) for hyperactive/impulsive symptoms, and 9% (n=5) for combined symptoms of ADHD). There were significant associations with estimated IQ (SDQ rho=-.44, p<.001; inattention rho=-.45, p=.001; hyperactive/impulsive rho=-.33, p=.02). Clinician-rated best-estimate diagnosis indicated that 15% (n=12) participants met criteria for definite ADHD and a further 26% (n=21) met criteria for possible ADHD. There was a significant group difference in IQ ($F(2, 75) = 4.86$, p=.01); participants not meeting clinical ADHD criteria had higher IQ (76.68) compared to definite cases (57.25, p=.03).

Longitudinal pathways to ADHD symptoms in TSC

Bivariate correlations between variables and ADHD symptoms and further information on epilepsy characteristics are provided in the Supplementary Material (Section 2). There were no significant indirect pathways to DAWBA-rated hyperactive/impulsive symptoms; findings are not reported. All significant path coefficients are shown in Figures 1 for SDQ-rated ADHD symptoms and Figure 2 for DAWBA-rated inattention symptoms.

Two indirect paths were significant for SDQ-rated ADHD symptoms (Figure 1), through: (1) mutation type (TSC2 versus TSC1), to increased tuber load, to increased spasm severity to increased ADHD symptoms (β =0.28, 95% CI 0.03, 0.72); and (2) mutation type, to tuber load, to increased non-spasm seizure severity, to increased current seizure severity to ADHD symptoms (β =0.20, 95% CI 0.06, 0.50).

<FIGURE 1>

One significant indirect path was indicated between mutation and DAWBA-rated inattention symptoms (Figure 2), observed through: mutation type, to tuber load, to increased non-spasm seizure severity, to increased current seizure severity, to ADHD symptoms (β =0.14, 95% CI 0.03, 0.44).

<FIGURE 2>

To control for intellectual ability, current estimated IQ was added into the models as a correlated factor. The significant pathways to ADHD were retained, while pathways to IQ replicated our previous findings (Supplementary Figures 1,2).

DISCUSSION

This study aimed to characterise the interdependence of clinical features of TSC on the developmental pathway to ADHD symptoms in a prospective longitudinal cohort study. While there was no difference in ADHD symptoms by type of TSC mutation in line with previous work[3], sophisticated structural equation modelling indicated a significant indirect pathway linking genetic mutation (TSC2), to increased tuber load, to increased severity of epileptic spasms, to increased SDQ-rated ADHD symptoms. This pathway suggests that the association between genetic mutation and ADHD symptoms in later childhood/adolescence is mediated by tuber burden and epileptic spasm severity in the early years. This is consistent with previous reports of increased ADHD in individuals with a history of spasms[4]. Given spasms are associated with more severe and intractable epilepsy[2], this suggests that early control of seizures is key for long-term behavioural outcomes.

An additional pathway was demonstrated, operating via tuber load, to increased severity of non-spasm seizures, to increased current seizure severity, to increased ADHD symptoms, both SDQ-rated and DAWBA-rated (inattention). There is considerable stability of seizure severity over time, and this might reflect the chronic impact of seizures over time culminating in higher ADHD symptoms. It is unknown whether characteristics of ADHD antedate onset of epilepsy or fluctuate with severity. The role of early seizure severity should therefore be systematically explored through repeated measurement from early in life prior to emergence of ADHD traits.

There were moderate correlations between ADHD symptoms and estimated IQ. When entering intellectual ability as a correlated outcome the indirect pathways were retained for both SDQ-rated ADHD and DAWBA-rated inattention symptoms. In this cohort, the pathway through early life non-spasm seizure severity to cognitive ability in middle childhood and adolescence was previously implicated[7], suggesting a potential distinction between pathways to later IQ and later ADHD in TSC. Although our findings suggest that risk pathways for ID and ADHD in TSC do not completely overlap, it is likely that reduced cognitive ability may influence symptoms of ADHD, and characteristics of ADHD may impact upon intellectual and adaptive skills, in a bidirectional way[12].

There are several challenges associated with measurement of ADHD in cognitively-impaired individuals. Although the selected measures may produce high prevalence scores in this population, their validity and reliability has previously been demonstrated[13]. Still it is unclear whether the symptoms measured represent ADHD in TSC, or difficulties associated with ID, and current antiepileptic medication and seizure severity may present as attentional difficulties. Direct comparison of ADHD in syndromic and non-syndromic individuals is required, alongside more objective measurement of activity[14] and neurocognitive impairment associated with ADHD, which may be independent of aetiological factors shared with IQ[15]. Because diagnostic overshadowing of ID may limit reports of additional behavioural challenges, it is also important to consider the role of

other TAND manifestations, including autism (Supplementary Material Section 2), mood and anxiety disorders[4].

In conclusion, the findings support a cascading developmental risk pathway linking the type of genetic mutation to neurological manifestations of TSC through to ADHD symptoms. Detailed investigation of seizure characteristics and cognitive and behavioural sequelae associated with ADHD in the first years of life is warranted.

Acknowledgements

We wish to thank all of the families for their time and help with this study. The members of the Tuberous Sclerosis 2000 Study Group are: V Attard, A Clarke, FV Elmslie, AK Saggar, St George's Hospital, London; D Baines, BA Kerr, Royal Manchester Children's Hospital, Manchester; C Brayne, Institute of Public Health, University of Cambridge; I Carcani-Rathwell, C Connolly, M Clifford, A Lydon, F Oluwo, H Rogers, C Srivastava, Institute of Psychiatry, Psychology & Neuroscience, King's College London; JA Cook, Sheffield Children's Hospital, Sheffield; C Falconer, St James's University Hospital, Leeds; DM Davies, JR Sampson, Institute of Medical Genetics, Cardiff; AE Fryer, Alder Hey Children's Hospital, Liverpool; M Haslop, Y Granader*, University of Cambridge (*currently Yeshiva University, New York); PD Griffiths, University of Sheffield; A Hunt, Tuberous Sclerosis Association; WWK Lam, Western General Hospital, Edinburgh; JC Kingswood, Royal Sussex County Hospital, Brighton; ZH Miedzybrodzka, College of Life Sciences and Medicine, Aberdeen; H Crawford, PJ Morrison, Belfast City Hospital; FJK O'Callaghan, Great Ormond Street Hospital/Institute of Child Health, University College London; SG Philip, Birmingham Children's Hospital, Birmingham; S Seri, Aston Brain Centre, School of Life and Health Sciences, Aston University, Birmingham; R Sheehan-Dare, The General Infirmary, Leeds; CH Shepherd, Craigavon Area Hospital, Craigavon.

Author contributions

CT designed the study, acquired, analysed and interpreted the data and drafted the manuscript. FSM designed the study, acquired the data and revised the manuscript. HL, EW, LU, ES acquired the data and revised the manuscript. EDB interpreted the data and revised the manuscript. FS, NH, JS analysed the data and revised the manuscript. PFB designed the study, interpreted the data and revised the manuscript. All authors approved the final version of the manuscript.

Data availability statement

Readers seeking access to this data should contact Dr Charlotte Tye, King's College London (charlotte.tye@kcl.ac.uk). Access to restricted and fully anoymised data will be granted to named individuals in accordance with research governance procedures governing the reuse of data. Specifically, requestors must complete a study data access request form with details of proposed data usage and a formal data sharing agreement. This may require additional ethical approval depending on the nature of the request.

Funding statement

This study was supported by grants awarded to Patrick Bolton from the Baily Thomas Charitable Fund, Tuberous Sclerosis Association (TSA) and Autism Speaks. Charlotte Tye was funded by a Medical Research Council studentship and supported by the Tuberous Sclerosis Association and the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London. The views expressed are those of the authors and not necessarily those of the NHS, NIHR or the Department of Health and Social Care. Elizabeth Shephard is supported by the Sao Paulo Research Foundation (FAPESP; grant number 2020/05964-1).

Conflict of interest disclosure

None of the authors has any conflict of interest to disclose.

Ethical publication statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

REFERENCES

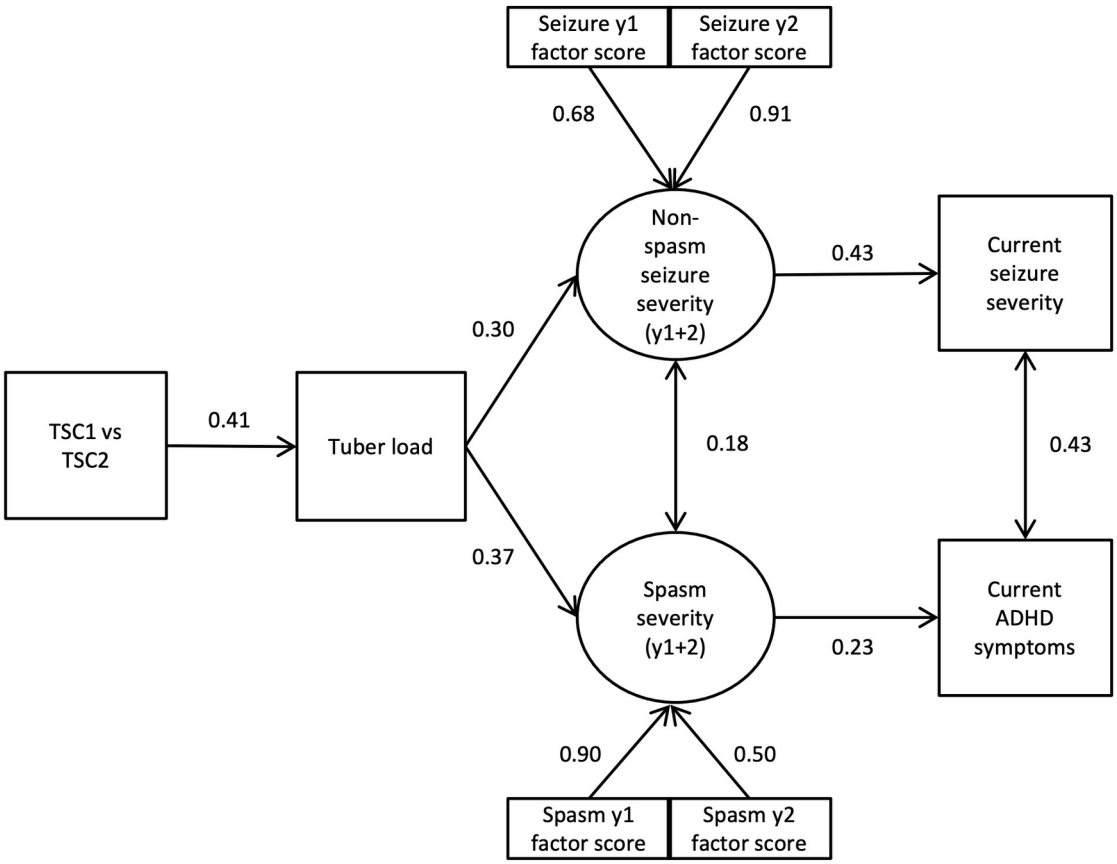
1. Henske EP, Jóźwiak S, Kingswood JC, Sampson JR, Thiele EA. Tuberous sclerosis complex. *Nature reviews Disease primers* 2016;2:1-18.

2. Chu-Shore CJ, Major P, Camposano S, Muzykewicz D, Thiele EA. The natural history of epilepsy in tuberous sclerosis complex. *Epilepsia* 2010;51:1236-1241.
3. De Vries PJ, Belousova E, Benedik MP, Carter T, Cottin V, Curatolo P, Dahlin M, D'Amato L, d'Augères GB, Ferreira JC. TSC-associated neuropsychiatric disorders (TAND): findings from the TOSCA natural history study. *Orphanet journal of rare diseases* 2018;13:1-13.
4. Muzykewicz DA, Newberry P, Danforth N, Halpern EF, Thiele EA. Psychiatric comorbid conditions in a clinic population of 241 patients with tuberous sclerosis complex. *Epilepsy & Behavior* 2007;11:506-513.
5. de Vries PJ, Hunt A, Bolton PF. The psychopathologies of children and adolescents with tuberous sclerosis complex (TSC). *European Child & Adolescent Psychiatry* 2007;16:16-24.
6. D'Agati E, Moavero R, Cerminara C, Curatolo P. Attention-deficit hyperactivity disorder (ADHD) and tuberous sclerosis complex. *Journal of Child Neurology* 2009;24:1282-1287.
7. Tye C, Mcewen FS, Liang H, Underwood L, Woodhouse E, Barker ED, Sheerin F, Yates JRW, Bolton PF, Group TSS. Long-term cognitive outcomes in tuberous sclerosis complex. *Developmental Medicine & Child Neurology* 2020;62:322-329.
8. Pan P-Y, Bölte S. The association between ADHD and physical health: a co-twin control study. *Scientific Reports* 2020;10:1-13.
9. Gupta A, de Bruyn G, Tousseyn S, Krishnan B, Lagae L, Agarwal N, Minnesota Epilepsy G, Frost M, Sparagana S, LaJoie J, et al. Epilepsy and Neurodevelopmental Comorbidities in Tuberous Sclerosis Complex: A Natural History Study. *Pediatric neurology* 2020;106:10-16.
10. Yates JR, Maclean C, Higgins JN, Humphrey A, le Marechal K, Clifford M, Carcani-Rathwell I, Sampson JR, Bolton PF. The Tuberous Sclerosis 2000 Study: presentation, initial assessments and implications for diagnosis and management. *Arch Dis Child* 2011;96:1020-1025.
11. Humphrey A, Ploubidis GB, Yates JR, Steinberg T, Bolton PF. The early childhood epilepsy severity scale (E-chess). *Epilepsy research* 2008;79:139-145.
12. Rommel AS, Rijsdijk F, Greven CU, Asherson P, Kuntsi J. A longitudinal twin study of the direction of effects between ADHD symptoms and IQ. *PloS one* 2015;10:e0124357.
13. Emerson E. Use of the Strengths and Difficulties Questionnaire to assess the mental health needs of children and adolescents with intellectual disabilities. *Journal of Intellectual and Developmental Disability* 2005;30:14-23.
14. Earnest T, Shephard E, Tye C, McEwen F, Woodhouse E, Liang H, Sheerin F, Bolton PF. Actigraph-Measured Movement Correlates of Attention-Deficit/Hyperactivity Disorder (ADHD) Symptoms in Young People with Tuberous Sclerosis Complex (TSC) with and without Intellectual Disability and Autism Spectrum Disorder (ASD). *Brain sciences* 2020;10:491.
15. Wood A, Rijsdijk F, Johnson K, Andreou P, Albrecht B, Arias-Vasquez A, Buitelaar JK, McLoughlin G, Rommelse N, Sergeant JA. The relationship between ADHD and key cognitive phenotypes is not mediated by shared familial effects with IQ. *Psychological medicine* 2011;41:861-871.

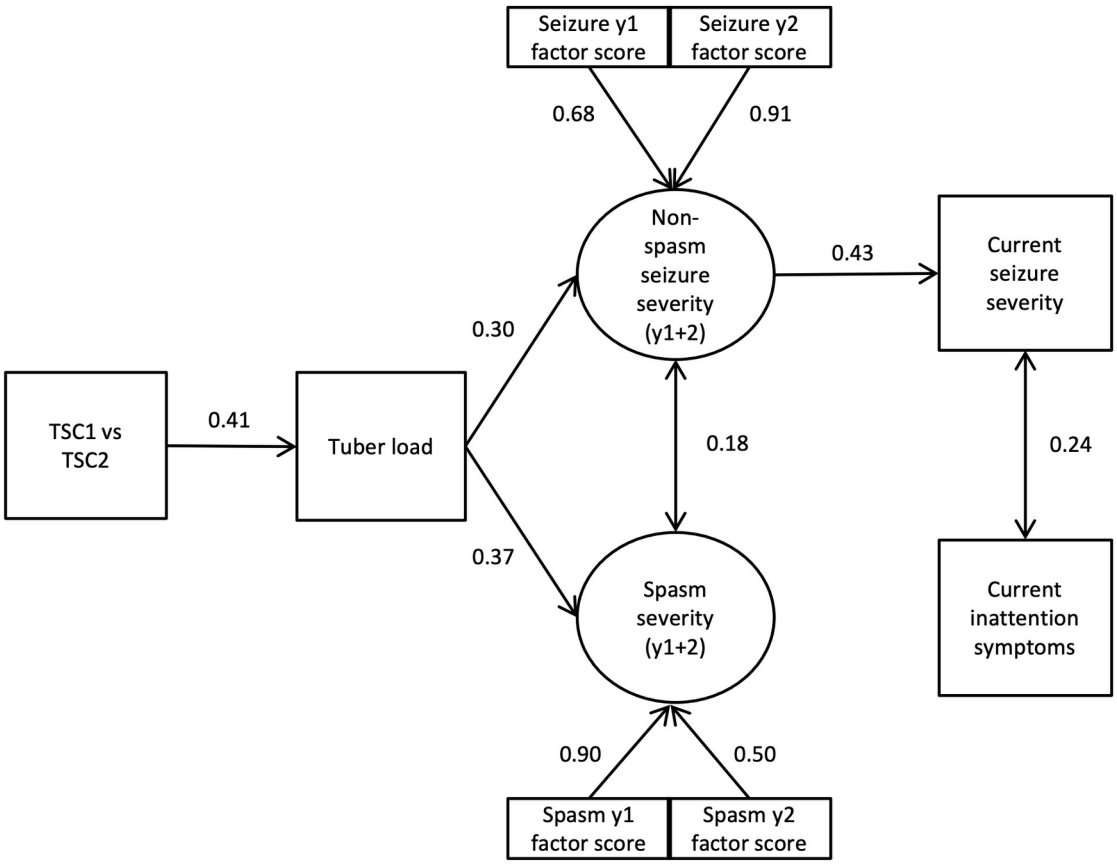
FIGURES

FIGURE 1: Full mediation model for SDQ hyperactivity: Paths linking genotype and SDQ hyperactivity scores, through tuber load and epilepsy severity. Ovals represent latent variables and rectangles represent observed variables. Only significant paths are shown; absence of a line connecting variables implies no direct effect (path was not significant). Standardised betas for each path are shown, all paths shown are significant at $p<.05$. Model fit: RMSEA=0.07 (90% CI=0.01-0.11); standardized RMR=0.06, CFI=0.95.

FIGURE 2: Full mediation model for DAWBA DSM-5 inattention symptom count: Paths linking genotype and inattention symptoms, through tuber load and epilepsy severity. Ovals represent latent variables and rectangles represent observed variables. Only significant paths are shown; absence of a line connecting variables implies no direct effect (path was not significant). Standardised betas for each path are shown, all paths shown are significant at $p<.05$. Model fit: RMSEA=0.05 (90% CI=0.01-0.10); standardized RMR=0.07, CFI=0.97.



EPI_17507_Tye_Figure 1.tiff



EPI_17507_Tye_Figure 2.tiff