

Human *GABRG2* generalized epilepsy

Increased somatosensory and striatothalamic connectivity

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Abstract

Objective

To map functional MRI (fMRI) connectivity within and between the somatosensory cortex, putamen, and ventral thalamus in individuals from a family with a GABAergic deficit segregating with febrile seizures and genetic generalized epilepsy.

Methods

We studied 5 adults from a family with early-onset absence epilepsy and/or febrile seizures and a GABA_A receptor subunit gamma2 pathogenic variant (*GABRG2*[R43Q]) vs 5 age-matched controls. We infer differences between participants with the *GABRG2* pathogenic variant and controls in resting-state fMRI connectivity within and between the somatosensory cortex, putamen, and ventral thalamus.

Results

We observed increased fMRI connectivity within the somatosensory cortex and between the putamen and ventral thalamus in all individuals with the *GABRG2* pathogenic variant compared with controls. Post hoc analysis showed less pronounced changes in fMRI connectivity within and between the primary visual cortex and precuneus.

Conclusions

Although our sample size was small, this preliminary study suggests that individuals with a *GABRG2* pathogenic variant, raising risk of febrile seizures and generalized epilepsy, display underlying increased functional connectivity both within the somatosensory cortex and in striatothalamic networks. This human network model aligns with rodent research and should be further validated in larger cohorts, including other individuals with generalized epilepsy with and without known GABA pathogenic variants.

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Glossary

CI = confidence interval; fMRI = functional MRI.

We previously reported an Australian family with a GABA type A receptor subunit gamma2 pathogenic variant (*GABRG2*[R43Q]) presenting with febrile seizures, febrile seizures plus, and absence epilepsy.¹ This family has decreased cortical inhibition, demonstrated by transcranial magnetic stimulation,² and decreased cortical benzodiazepine receptor binding on PET.³ Aligning with human data, mice with the same *Gabrg2*[R43Q] pathogenic variant have reduced cortical inhibition shown by deficits in GABA-mediated synaptic currents in the somatosensory cortex and anatomic changes in cortical interneuron positioning.^{4,5}

Although these investigations provide clues about the mechanisms underlying the *GABRG2* pathogenic variant, it is unknown whether brain connectivity is affected in humans with the R43Q pathogenic variant. To study this issue, we quantified changes in functional MRI (fMRI) connectivity in individuals with this *GABRG2* pathogenic variant. We focused on regions previously studied in *Gabrg2* mice: the somatosensory cortex, the ventral thalamus, and the putamen (as a recent fMRI study in rats demonstrated that GABA_A antagonists enhance connectivity of the somatosensory cortex and striatum⁶). Together, these 3 brain regions comprise a well-known pathway where the cortex sends excitatory signals to the putamen, which in turn exerts strong inhibitory control over the ventral thalamus via the globus pallidus and substantia nigra pars reticulata before relaying excitatory information back to the cortex.

Based on previous research in humans with the *GABRG2* [R43Q] pathogenic variant^{2,3} and mice with the *Gabrg2*[R43Q] pathogenic variant,^{4,5,7} we hypothesize that fMRI connectivity within the somatosensory cortex and between subcortical regions is altered in people with a *GABRG2* pathogenic variant.

Methods

Participants and clinical information

We recruited 5 adults from a previously reported family with the *GABRG2* [R43Q] pathogenic variant¹ (mean age [SD]: 36.4 ± 4.2 years; clinical information in the table). They were compared with 5 age- and sex-matched controls (mean age [SD] 36.8 ± 4.1 years).

Standard protocol, approvals, and patient consents

This study was approved by the Austin Human Research Ethics Committee, and participants gave written informed consent to participate.

fMRI preprocessing

We acquired 10 minutes of resting-state fMRI data on a Siemens 3T Skyra scanner with a voxel size of 3 × 3 × 3 mm and repetition time of 3 seconds. The fMRI data were slice-timing corrected, realigned, coregistered to T1 images, tissue segmented, spatially normalized, and filtered between 0.01 and 0.08 Hz. See reference 8 for further details about our fMRI methods.

fMRI connectivity analysis

We used 2 different estimates of fMRI connectivity. (1) Regional homogeneity: to calculate voxel-averaged local connectivity within each node of our brain network model (somatosensory cortex, ventral thalamus, and putamen). This measures Kendall *W* correlations between a single voxel and its 26 nearby voxels in three-dimensional space. Its values range between 0 (minimal local connectivity) and 1 (maximal local connectivity). (2) Partial correlation: to calculate voxel-averaged Pearson correlations of fMRI time series between each node pair, while regressing out indirect correlation effects from all other connection pairs. Partial

Table Clinical, EEG and imaging phenotype of individuals with the R43Q *GABRG2* pathogenic variant¹

Patient no.	Sex	Age (y)	Onset	Offset	Seizure type	EEG	Syndrome	MRI	AEDs	<i>GABRG2</i> pathogenic variant
1	F	41	Infant	Early teens	FS, AS	GSW	CAE	Normal	0	Positive
2	F	33	Infant	Ongoing	FS, AS	GSW	CAE	Normal	0	Positive
3	M	31	Infant	Toddler	FS	Normal	FS only	Normal	0	Positive
4	M	39	Infant	36 y	FS, AS, GTCS, FIAS	GSW, L-TIRDA	EOAE (childhood), L-TLE (adulthood)	Normal	1 (CBZ)	Positive
5	M	38	Infant	Toddler	FS	Normal	FS only	Normal	0	Positive

Abbreviations: AED = current antiepileptic drug; AS = absence seizure; CAE = childhood absence epilepsy; CBZ = carbamazepine; EOAE = early-onset absence epilepsy; FIAS = focal impaired awareness seizure; FS = febrile seizure; GTCS = generalized tonic-clonic seizure; GSW = generalized spike-wave discharge; L-TLE = left temporal lobe epilepsy; L-TIRDA = left temporal intermittent rhythmic delta activity.

correlation values range between -1 (maximal negative correlation) and 1 (maximal positive correlation).

Effect size analysis

We used Hedges' g standardized effect sizes and 95th percentile confidence intervals (95% CIs) to quantify mean differences in brain connectivity between individuals with the *GABRG2* pathogenic variant and controls (as Hedges' g is recommended over Cohen's d in studies with a limited sample sizes). Hedges' g effect sizes = 0.2 (small); 0.5 (moderate); 0.8 (large); and 1.2 (very large).

Data availability

Anonymized original data will be shared by request from any qualified investigator.

Results

GABRG2: fMRI connectivity is increased within the somatosensory cortex

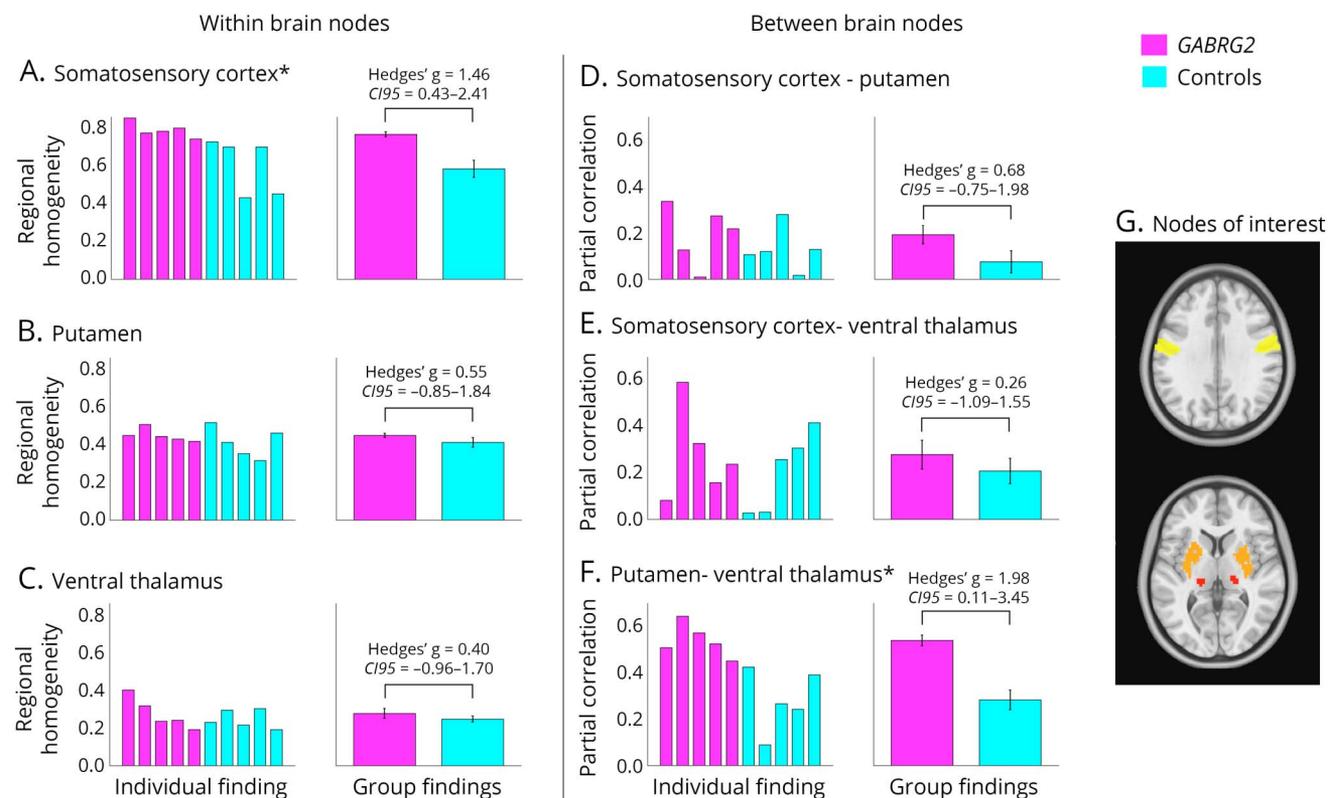
We observed stronger fMRI connectivity within the somatosensory cortex in all individuals with the *GABRG2* pathogenic

variant compared with controls (Hedges' $g = 1.46$; 95% CI = 0.43–2.41—figure, A). Hedges' g effect sizes were lower between *GABRG2* and healthy controls for fMRI connectivity within the putamen (Hedges' $g = 0.55$; CI 95 = -0.85 – 1.84) and ventral thalamus (Hedges' $g = 0.40$; CI 95 = -0.96 to 1.70).

GABRG2: fMRI connectivity is increased between the thalamus and putamen

We observed stronger fMRI connectivity between the putamen and ventral thalamus in all individuals with the *GABRG2* pathogenic variant compared with controls (Hedges' $g = 1.98$; 95% CI = 0.11–3.45—figure, F). It is worth noting that the *GABRG2* participant with strongest connectivity also experienced seizures into adulthood (see participant 2 in the table for more clinical information). There were no large effect sizes between *GABRG2* and controls for fMRI connectivity between the putamen and somatosensory cortex (Hedges' $g = 0.68$; 95% CI = -0.75 – 1.98) or the ventral thalamus and somatosensory cortex (Hedges' $g = 0.26$; 95% CI = -1.09 to 1.55).

Figure Functional connectivity within and between brain nodes in *GABRG2* participants displayed by purple magenta color bars and healthy controls displayed by blue cyan color bars



Peak regional homogeneity across all voxels within the somatosensory cortex (A), putamen (B), and ventral thalamus (C), and partial correlations averaged across all voxels between the somatosensory cortex-putamen (D), somatosensory cortex-ventral thalamus (E), and ventral thalamus-putamen (F). Error bars denote the standard error across participants. There was little difference in head movement between groups (mean movement [SD] in *GABRG2* = 0.12 mm \pm 0.05; mean movement [SD] in controls = 0.16 mm \pm 0.04). Our 3 brain regions of interest—(1) bilateral somatosensory cortex (yellow); (2) putamen (orange); and (3) ventral thalamus (red) (G)—were delineated using the NeuroSynth database (neurosynth.org) incorporating findings from a multitude of previous fMRI studies describing these brain regions and their behavioral correlates, as well as their functional connectivity patterns. *A and F had large effect size.

GABRG2: post hoc analysis shows less fMRI connectivity changes of the visual cortex and precuneus

To test whether our primary result was specific to the somatosensory cortex, thalamus, and putamen or it reflected a “whole-brain process”, we analyzed 2 additional brain nodes: (1) primary visual cortex and (2) precuneus. We observed no large effect size in fMRI connectivity for these 2 brain regions between *GABRG2* and control participants. fMRI connectivity within the visual cortex: Hedges’ $g = 0.45$; 95% CI = -0.93 to 1.74 . fMRI connectivity within the precuneus: Hedges’ $g = 0.25$; 95% CI = -1.12 to 1.56 . fMRI connectivity between the visual cortex and precuneus: Hedges’ $g = 0.43$; 95% CI = -0.95 – 1.72 .

Discussion

We found that 5 individuals with a *GABRG2* pathogenic variant and a history of seizures (5/5 febrile seizures; 3/5 absence seizures) have increased fMRI connectivity within the somatosensory cortex and between the putamen and ventral thalamus compared with 5 healthy controls. This finding suggests a “network-specific” rather than a “whole-brain” effect, as our post hoc analysis revealed no difference in connectivity between *GABRG2* and control participants of either the primary visual cortex or the precuneus.

Emerging animal research has highlighted the important role of specific brain areas in generalized epilepsy, in particular absence epilepsy where seizures are thought to originate in the somatosensory cortex⁹ and are modulated by thalamostriatal circuits.¹⁰ Although it is not straightforward to compare animal and human studies, our findings provide preliminary evidence that somatosensory cortex and subcortical structures are hyperconnected in people with a genetic predisposition to develop febrile and also absence seizures. This finding is further supported by our previous *GABRG2* transcranial magnetic stimulation study—in the same family—showing neuronal hyperexcitability of the perimotor cortex.²

It is nontrivial to quantify whether microscale neuronal dysfunction and macroscale fMRI connectivity are related because of their vast difference in spatial scales. However, this study presented us with an opportunity to (indirectly) assess whether inhibitory neuronal dysfunction is reflected in fMRI connectivity, as we know that people with a *GABRG2* pathogenic variant have abnormal inhibitory GABAergic neuronal function. We postulate that our fMRI connectivity findings are related to inhibitory neuronal abnormalities, especially hyperconnectivity between the thalamus and the putamen, as the latter brain region consists almost exclusively of medium spiny inhibitory GABAergic neurons.

Our small sample size is a consequence of the difficulty of recruiting a single family with a genetically homogenous

disorder to the demands of an imaging study that requires travel and attendance at a single site. Despite this limitation, our preliminary findings give insight into network changes that may underlie human genetic epilepsy caused by a *GABRG2* pathogenic variant, and they align with results from the *Gabrg2* animal model, which also shows increased activity of the somatosensory cortex.⁴ Nevertheless, our human network model based on this family with a *GABRG2* variant should be further validated in larger generalized epilepsy cohorts with and without known GABA pathogenic variants.

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Disclosure

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Name	Location	Role	Contribution
Mangor Pedersen, PhD	The Florey Institute of Neuroscience and Mental Health	Author	Conception and design of the study, acquisition and analysis of data, and writing the first draft of the manuscript
Magdalena Kowalczyk, MSc	The Florey Institute of Neuroscience and Mental Health	Author	Conception and design of the study and acquisition and analysis of data
Amir Omidvarnia, PhD	The Florey Institute of Neuroscience and Mental Health	Author	Conception and design of the study and analysis of data
Piero Perucca, MD, PhD	The University of Melbourne	Author	Conception and design of the study and acquisition and analysis of data

Appendix *(continued)*

Name	Location	Role	Contribution
Samuel Gooley, MBBS	The University of Melbourne	Author	Conception and design of the study and acquisition and analysis of data
Steven Petrou, PhD	The Florey Institute of Neuroscience and Mental Health	Author	Conception and design of the study and codesigned and drafted a significant portion of the manuscript
Ingrid E. Scheffer, MBBS, PhD	The University of Melbourne	Author	Conception and design of the study and codesigned and drafted a significant portion of the manuscript
Samuel F. Berkovic, MD	The University of Melbourne	Author	Conception and design of the study and codesigned and drafted a significant portion of the manuscript
Graeme D. Jackson, MD	The Florey Institute of Neuroscience and Mental Health	Author	Conception and design of the study and drafted a significant portion of the manuscript

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