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Perisylvian and Hippocampal Anomalies in Individuals With Pathogenic *GRIN2A* Variants

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Abstract

Background and Objectives

Pathogenic variants in *GRIN2A* are associated with a spectrum of epilepsy-aphasia syndromes (EASs). Seizures as well as speech and language disorders occur frequently but vary widely in severity, both between individuals and across the life span. The link between this phenotypic spectrum and brain characteristics is unknown. Specifically, altered brain networks at the root of speech and language deficits remain to be identified. Patients with pathogenic variants in *GRIN2A* offer an opportunity to interrogate the impact of glutamate receptor dysfunction on brain development.

Methods

We characterized brain anomalies in individuals with pathogenic *GRIN2A* variants and EASs, hypothesizing alterations in perisylvian speech-language regions and the striatum. We compared structural MRI data from 10 individuals (3 children and 7 adults, 3 female) with pathogenic *GRIN2A* variants with data from age-matched controls (N = 51 and N = 203 in a secondary analysis). We examined cortical thickness and volume in 4 a priori hypothesized speech and language regions (inferior frontal, precentral, supramarginal, and superior temporal) and across the whole brain. Subcortical structures (hippocampus, basal ganglia, thalamus) and the corpus callosum were also compared.

Results

Individuals with pathogenic *GRIN2A* variants showed increased thickness and volume in the posterior part of Broca's area (inferior frontal gyrus, pars opercularis). For thickness, the effects were bilateral but more pronounced in the left (large effect size, $\eta^2 = 0.37$) than the right ($\eta^2 = 0.12$) hemisphere. Both volume and thickness were also higher in the bilateral superior temporal region while the supramarginal region showed increased thickness only. Whole-brain analyses confirmed left-sided thickness increases in Broca's area, with additional increases in the occipital and superior frontal cortices bilaterally. Hippocampal volume was reduced in the left hemisphere. There were no age-dependent effects or corpus callosum group differences.

Discussion

Anomalies in perisylvian regions, with largest differences in Broca's area, suggest an altered development of classical speech-language networks in *GRIN2A*-related EAS. Left hippocampal reduction suggests a role for this structure in early speech and language development and is consistent with *GRIN2A* gene expression in that region. Overall, elucidating the neural correlates of EAS provides insights into the impact of *GRIN2A* dysfunction, opening avenues for targeted intervention in developmental syndromes with compromised speech-language development.

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Glossary

CAS = childhood apraxia of speech; **EAS** = epilepsy-aphasia syndrome; **TICV** = total intracranial volume.

Introduction

GRIN2A encodes the GluN2A protein, a subset of NMDA receptors involved in brain development, synaptic plasticity, and learning.[1] In humans, pathogenic variants of *GRIN2A* are associated with epilepsy-aphasia syndromes (EASs; see review 2-5) with onset in childhood, including Landau-Kleffner syndrome and Rolandic epilepsy.[4] Speech-language impairments range from absent to severe [2,6] and are not always associated with the presence of seizures.[6,7] Speech disorders primarily manifest as dysarthria and speech dyspraxia (now childhood apraxia of speech, CAS) with oral motor impairments. Language regression is present for many and varies in association with seizure type.[7] Intellectual disability is common (62% in the study by Strehlow et al.[6]) but mostly mild. Despite a well-described behavioral and neurologic phenotype, neuroimaging markers of *GRIN2A* syndrome have not been identified. Advanced MRI analysis techniques now allow us to detect subtle brain anomalies that can elucidate the association between pathogenic *GRIN2A* variants and clinical phenotypes.

Few studies have examined MRI profiles in pathogenic *GRIN2A* variants. Data from unrelated individuals suggest most have "normal" clinical MRI scans[8] (75% in a study with n = 85).[6] There is limited evidence of visible subcortical and cortical anomalies. Individuals may show brain atrophy (11%[6]) while others show regional cortical dysplasia, reduced corpus callosum, or hippocampal sclerosis (e.g., a few cases in the study by Strehlow et al.[6]; see the study by Pierson et al.[9] for a case with general hypomyelination at age 9 years). It is noteworthy that MRI profiles may also depend on genotypes[6] and that milder gain-of-function variations are linked to milder phenotypes.[10] Finally, a study of 144 healthy individuals reported enlarged hippocampi and amygdalae in those with short allele carriers of *GRIN2A* (n = 89) than in those with homozygous long alleles (n = 55), but no whole-brain group differences.[11] Overall, the link between these diverse brain anomalies and speech-language profiles remains to be elucidated in the context of pathogenic *GRIN2A* variants.

Postmortem studies in humans indicate that *GRIN2A* is expressed in the dorsolateral prefrontal cortex,[12] hippocampal formation, amygdala, basal ganglia, and thalamus[13,14] as well as the cerebellum.[12] Mice knock-out models of *Grin2a* show anomalies in the neocortex as well as in the hippocampus, corpus callosum, and thalamus.[15] Similarly, mice carrying putative gain-of-function variants display thinning of hippocampal structures early in development.[16] Overall, the development of both neocortical and subcortical structures could, therefore, be affected by pathogenic *GRIN2A* variants in humans.

Recent advances in MRI analysis techniques have revealed novel neural phenotypes in genetic conditions where speech and language disorders are observable early in development and persist into adulthood, as seen for *GRIN2A*. Structural and/or functional anomalies in the inferior frontal gyrus (Broca's area), superior temporal, and temporoparietal regions have been reported in a family with inherited CAS,[17] a boy with a severe speech-language phenotype due to a pathogenic *FOXP2* variant,[18] and in children with idiopathic CAS.[19] There is also strong evidence of disruption to a "speech execution" network (ventral primary motor cortices and corticobulbar tracts) in childhood-acquired dysarthria (e.g., after brain injury[20]) and idiopathic articulation disorders.[21] At the subcortical level, volumetric reductions in the caudate nucleus, hippocampus, and thalamus have been reported in children and adults with *FOXP2*-related CAS.[18,22] On the contrary, increased volumes were found in the putamen[23] and were also observed in other instances of persistent CAS.[17] We know that most known pathogenic *GRIN2A* variants are inherited (60.2%[6]), yet the lack of MRI studies in families limits our understanding of genotype-phenotype associations.

We examined cortical thickness and volume as well as subcortical volumes in 10 participants with epilepsy-aphasia syndrome and pathogenic *GRIN2A* variants from 3 families. These individuals presented with language, speech, and intellectual impairments of varying degrees. We hypothesized cortical anomalies in perisylvian speech-language regions, namely the inferior frontal gyrus (Broca's area), superior temporal gyrus, and supramarginal gyrus, relative to age-matched control data. We also predicted alterations in the ventral precentral gyrus (primary motor cortex) due to dysarthric and oromotor features. In subcortical structures, we hypothesized volumetric differences in the striatum.

Methods

Participants

Participants With Pathogenic *GRIN2A* Variants

Ten individuals (7 adults, 3 children) with *GRIN2A* splice site and missense pathogenic variants and epilepsy-aphasia syndrome (EAS) from 3 families (Family A, n = 6; Family B, n = 3; Family C, one proband) were recruited (see Table 1 for clinical and genetic data and Figure 1 for pedigrees).

Standard Protocol Approvals, Registrations, and Patient Consents

All 3 families consented under the Human Research Ethics Committee at the Royal Children's Hospital, Melbourne, Project number #37353. Ethics approval for adult control data was obtained from the Austin Health Human Research Ethics Committee (#2012.04475). All MRI scans were obtained as part of a research protocol.

Table 1 Clinical and Genetic Data From Families B and C

Family	Sex	Speech severity	CAS/ Dysarthria	Epilepsy	Transcript#	Coding change	Protein change	Variant type	ACMG[a]
AC-IV-5	Male	Mildly impaired	CAS and Dysarthria	ADRESD	NM_ 001134407.3	c.1007+1G>A	N/A	Splice site	Pathogenic (PVS1, PP5, PM2)[b]
AC-III-5	Male	Mildly impaired	CAS and Dysarthria	ADRESD	NM_ 001134407.3	c.1007+1G>A	N/A	Splice site	
AC-III-2	Female	Mildly impaired		ADRESD	NM_ 001134407.3	c.1007+1G>A	N/A	Splice site	
AC-IV-2	Male	Mildly impaired	CAS and Dysarthria	ADRESD	NM_ 001134407.3	c.1007+1G>A	N/A	Splice site	
AC-V-1	Male	Moderately impaired	CAS and Dysarthria	ECSWS	NM_ 001134407.3	c.1007+1G>A	N/A	Splice site	
AC-IV-7	Male	Mildly impaired	CAS and Dysarthria	ECSWS	NM_ 001134407.3	c.1007+1G>A	N/A	Splice site	
B-II-1	Male	Moderately impaired	CAS	Focal epilepsy	NM_ 001134407.3	c.2138T>G	p.Val713Gly	Missense	Likely pathogenic (PP3, PM1, PM2, PP5)[c]
B-I-1	Female	Mildly impaired	CAS and Dysarthria	Mild focal epilepsy (BECTS-like)	NM_ 001134407.3	c.2138T>G	p.Val713Gly	Missense	
B-II-2	Male	Moderately impaired	CAS	ECSWS	NM_ 001134407.3	c.2138T>G	p.Val713Gly	Missense	
C	Female	Mildly impaired	Dysarthria	ECSWS	NM_ 001134407.3	c.2191G>A	p.Asp731Asn	Missense	Pathogenic (PP5, PS3, PP3, PM1, PM2)[d]

[a] American College of Medical Genetics (ACMG) criteria generated using VarSome.[24]
[b] ACMG criteria: c.1007+1G>A. PVS1 (Very Strong)—Null variant (intronic within ±2 of splice site) in gene GRIN2A. Loss-of-function is a known mechanism of disease (gene has 134 reported pathogenic LOF variants). PP5 (Very Strong)—ClinVar classifies this variant as Pathogenic, 2 stars (multiple consistent, reviewed Sep '23, 10 submissions), citing 9 articles. PM2 (Supporting)—Variant not found in gnomAD genomes or exomes.
[c] ACMG criteria: c.2138T>G (p.Val713Gly). PP3 (Strong)—MetaRNN = 0.943 is greater than 0.939 ⇒ strong pathogenic. PM1 (Supporting)—Hot-spot of length 17 amino-acids has 10 missense/in-frame variants (4 pathogenic variants, 5 uncertain variants and 1 benign variant), which qualifies as supporting pathogenic. PM2 (Supporting)—Variant not found in gnomAD genomes or exomes. PP5 (Supporting)—ClinVar classifies this variant as Uncertain Significance, 1 star (criteria provided, reviewed Aug '23, 3 submissions), citing 1 article, associated with Landau-Kleffner Syndrome, with 3 submissions (2 LP and 1 VUS).
[d] ACMG criteria: c.2191G>A (p.Asp731Asn). PP5 (Very Strong)—ClinVar classifies this variant as Pathogenic, 2 stars (multiple consistent, reviewed Sep '23, 4 submissions), citing 2 articles. PS3 (Strong)—UniProt Variants classifies this variant as Pathogenic, backed by functional studies (requires user validation) mentioned in 3 articles, associated with Epilepsy, focal, with speech disorder and with or without impaired intellectual development and Landau-Kleffner syndrome. PP3 (Strong)—MetaRNN = 0.946 is greater than 0.939 ⇒ strong pathogenic. PM1 (Moderate)—Hot-spot of length 17 amino-acids has 10 missense/in-frame variants (4 pathogenic variants, 6 uncertain variants and no benign), which qualifies as moderate pathogenic. UniProt protein NMDE1_HUMAN binding site 'Other binding site_730–731' has 3 missense/in-frame variants (2 pathogenic variants, 1 uncertain variant and no benign), which qualifies as moderate pathogenic. PM2 (Supporting) - Variant not found in gnomAD genomes or exomes.

Control Participants

Our primary analysis compared data from participants with pathogenic *GRIN2A* variants with 51 age and sex-matched controls scanned on the same 3.0T Siemens SKYRA scanner at the Brain Research Institute in Melbourne, Australia. Controls had no history of speech or language disorder, learning or cognitive difficulties, neurologic disorders, mental disorders, epilepsy, or seizures. Child control data were drawn from previous studies[20,21] with ethics approval from the Royal Children's Hospital Human Research Ethics Committee (#37353 and #31225). Ethics approval for adult control data was obtained from the Austin Health Human Research Ethics Committee (#2012.04475).

Owing to the limited availability of adult control data sets, the adult control group was smaller than the child control group. Therefore, we conducted a second analysis with 152 additional adult participants. We selected participants matched to our adult participants with pathogenic *GRIN2A* variants for age and sex from the Open Source IXI data set.[25] They were combined with the 51 control individuals scanned on the SKYRA scanner to form a control group of 203 participants. All results for this larger data set comparison are reported in Supplementary Material.

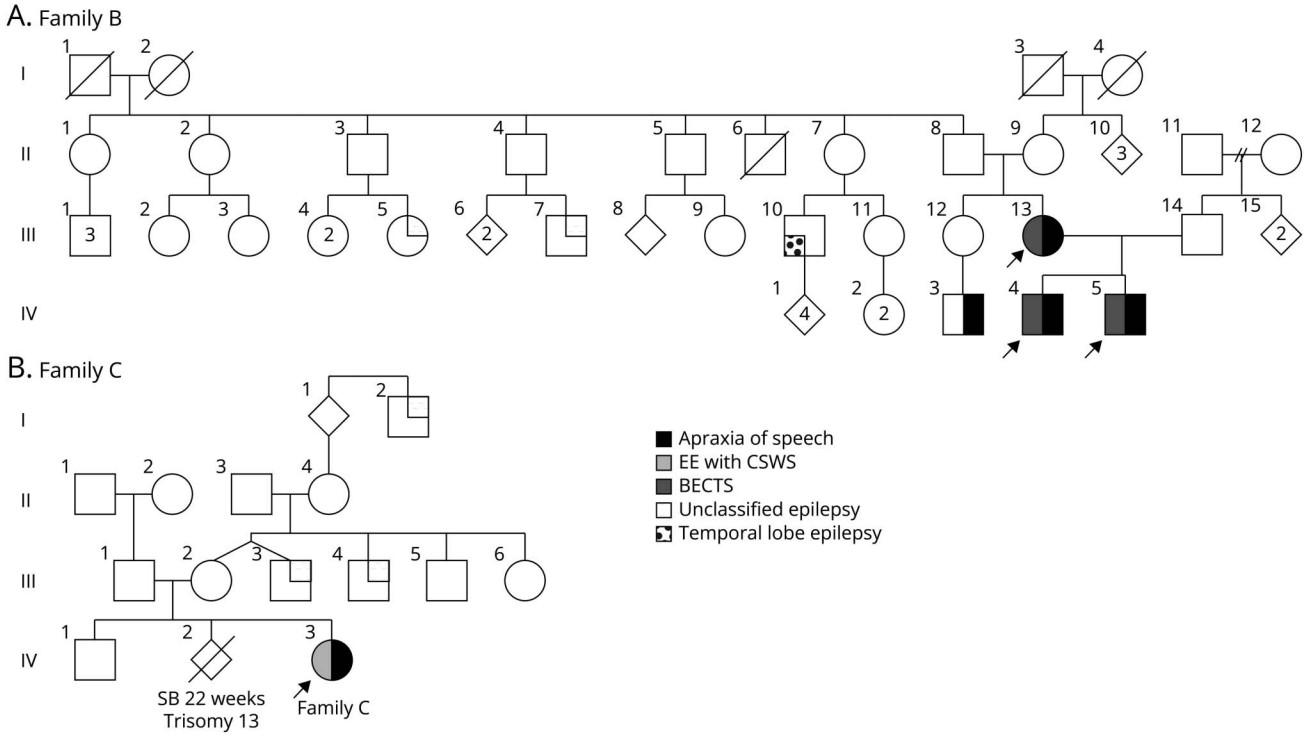
Brain MRI Acquisition and Processing

T1-weighted images were acquired from all participants. For the primary analyses (SKYRA), 160 T1-weighted images were obtained using an MP-RAGE sequence (TR = 1,900 ms, TE = 2.6 ms, flip angle = 9°, voxel size = 1 × 1 × 1mm^3). For the secondary analyses, IXI images had been collected from 3 scanners (London, UK): Hammersmith Hospital (Philips 3T), Guy's Hospital (Philips 1.5T), and the Institute of Psychiatry (GE 1.5T). Details of imagining parameters can be found at brain-development.org/ixi-dataset/.

Cortical Morphometry Reconstruction

All T1-weighted images were reconstructed using FreeSurfer 7.1.1 (surfer.nmr.mgh.harvard.edu/). Methods are described in full in the FreeSurfer documentation.[26-29] Briefly, FreeSurfer 7.1.1 includes intensity correction, skull stripping and

Figure 1 Pedigrees of Families B and C



Family A pedigree previously reported by Scheffer et al. (1995) and Turner (2015). Arrows indicate family members scanned. (A) Family B. Other disorders not depicted: B-II-11 psychosis, B-III-1, nemaline myopathy, B-III-2 depression anxiety, B-III-12 borderline personality disorder, B-III-11 MS, and B-IV-1 ASD. (B) Family C.

noise filtering, identification of white matter, separation of the hemispheres, and creation of a tessellated mesh representation of the white matter boundary and pial surface.

Data Analysis

As a priori hypothesized cortical regions of interest were specified, these were defined and extracted using the Desikan-Killiany atlas from FreeSurfer 'aparc' output. Subcortical and global volumes were obtained from the FreeSurfer 'aseg' output file.

For all analyses, data met the assumptions of GLMs run in SPSS v.27. We used nonparametric Mann-Whitney U tests when data did not meet the assumptions of the model. We compared global volumes (gray and white matter, CSF, and total intracranial) between groups using multivariate ANCOVA with age and sex as covariates.

Cortical Morphometry: ROI Analysis

We used a mixed-model multivariate ANCOVA to compare cortical thickness between groups in speech-language regions of interest (pars opercularis, precentral, superior temporal, and supramarginal regions) with hemispheric side as a within-subject variable and age and sex as covariates. The same model, with the inclusion of a group by age interaction, was then examined. Given that regional cortical volume (calculated as thickness X area) correlates with total intracranial volume (TICV),[27] we included TICV as an additional covariate in cortical volume MANCOVAs. For the secondary analysis (N = 203 controls), we ran the same MANCOVAs for both thickness and volume, with MRI scanner included as an additional covariate.

Cortical Morphometry: Whole-Brain Analysis

Whole-brain analyses were conducted on participants scanned on the SKYRA scanner only (*GRIN2A* = 10; controls = 51). As recommended for an exploratory analysis, we used a FWHM of 10 mm and resampled data to the fsaverage template. We fit a general linear model to the resampled data set to create group-level contrast, with significance level set at a minimum of $p < 0.005$ ($\log^{10}$ 2.3). To correct for false positives, we applied FreeSurfer's default Gaussian-Monte-Carlo simulation of 10,000 iterations. This was set with a vertex-wise threshold of $\log^{10}(1.3)$ (equivalent to $p = 0.05$). The significance threshold for clusters was set to $p = 0.025$, which corrects for analysis over both hemispheres.

Subcortical Analysis

For the thalamus, caudate, putamen, pallidum, hippocampus, and amygdala, left and right volumes were combined and calculated as a percentage of total intracranial volume to correct for age and head size. These corrected subcortical volumes were entered into a MANCOVA for group comparisons, with sex and age as covariates. For the secondary analysis (N = 203 controls), we ran the same MANCOVA with scanner included as an additional covariate.

Table 2 Speech and Language Profiles of Families B and C

Family	C	B-II-1	B-I-1	B-II-2
Receptive language score	71[a]	83[b]	Not assessed	57[b]
Expressive language score	71[a]	79[b]	Not assessed	61[b]
Core language score	75[a]	77[b]	Not assessed	55[b]
Reading	Not assessed	Third percentile[c]	Extremely low[c]	Extremely low[c]
Spelling	Not assessed	Third percentile[c]	Extremely low[c]	Extremely low[c]
Features of dysarthria	Slow speech rate, mildly slurred speech, reduced volume, monotone, prosodic difficulties, slow and laboured tongue movement, deviation of tongue to the left, slow movement of lips	Inappropriate silences, short rushes of speech, variable rate	Short rushes of speech, variable rate (also tongue fasciculations)	Speech features are more in line with CAS than dysarthria
Features of (C)AS	Increased errors with increased word length	Excess and equal stress, altered suprasegmental features, impaired syllable integrity, syllable segmentation, groping during speech and non-speech tasks, sound prolongations, mixed nasality, imprecise and frequent omission of consonants, vowel errors, schwa insertion, inconsistent errors	Excess and equal stress, altered suprasegmental features, impaired syllable integrity, syllable segmentation, groping during speech and non-speech tasks, mixed nasality, imprecise and frequent omission of consonants in connected speech, sound prolongations	Excess and equal stress, altered suprasegmental features, impaired syllable integrity, syllable segmentation, groping during speech and non-speech tasks, sound prolongations, mixed nasality, imprecise and frequent omission of consonants, vowel errors, voicing errors, inconsistent errors, epenthesis, non-phonemic sound distortions
Articulation disorder	+ Interdental Lisp	–	–	+ Lateral Lisp
Phonologic disorder	–	+	–	+
Phonologic delay	+	+	+	+
Dysarthria	+	+	+	–
CAS	–	+	+	+
Oromotor impairments	+	+	+	+

For Family A, see Carvill (2013)[3] and Turner (2015).[7].
[a] Scaled scores assessed with CELF-5[30] (mean = 100, SD = 15).
[b] Scaled scores assessed with CELF-4[31] (mean = 100, SD = 15).
[c] Data obtained from a previous speech and language report (assessment unknown).

Corpus Callosum Analysis

Total volume of the corpus callosum was calculated by summing all sections of the corpus callosum from the FreeSurfer output and dividing this by the total intracranial volume to correct for age and head size. This percentage was then entered into a univariate ANOVA for group comparison, with sex as a covariate.

Data Availability

Anonymized data not published within this article will be made available by reasonable request from any qualified investigator.

Results

Demographics

Owing to a larger number of child than adult controls, the *GRIN2A* group was older than the control group (*GRIN2A* mean (SD) = 380.1 (78.1) months; control mean (SD) = 197.1 (20.5) months; $t(59) = 2.26$, $p = 0.05$). Using the larger IXI control data set, the groups did not differ in age ($t(211) = -1.09$, $p = 0.27$).

Speech and Language Profiles

Family A's speech profiles have been comprehensively reported in previous publications.[3,7] Families C and B were not included in any previous speech and language phenotyping study and,

Figure 2 *GRIN2A* Participants Demonstrate Bilateral Increases in Cortical Thickness in the Pars Opercularis (*GRIN2A* n = 10; Controls n = 51)

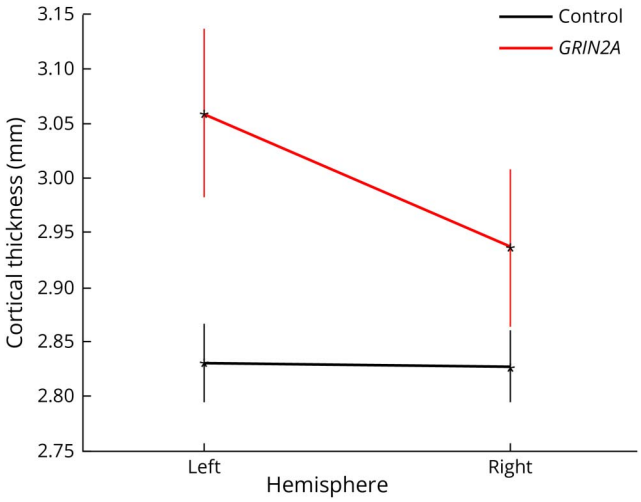


Figure shows estimated marginal means (controlling for age and sex). Group difference is significant in the left ($p < 0.0001$) and right ($p = 0.008$) hemispheres. Significant main effect of hemisphere and group*hemisphere interaction are also present.

therefore, underwent a speech and language assessment; the details are documented in Table 2. All *GRIN2A* participants had one or both motor speech disorders of CAS and dysarthria.

No Group Differences in Global Brain Volumes

There were no significant differences between *GRIN2A* and control groups in global volumes, including total gray matter ($F_{1,57} = 0.0$, $p = 0.99$, partial $\eta^2 = 0.00$), white matter ($F_{1,57} = 1.02$, $p = 0.32$, partial $\eta^2 = 0.02$), CSF ($F_{1,57} = 1.04$, $p = 0.742$, partial $\eta^2 = 0.00$), and estimated total intracranial ($F_{1,57} = 1.21$, $p = 0.28$, partial $\eta^2 = 0.02$) volumes. This lack of difference was confirmed between the *GRIN2A* group and the larger IXI control group for all measures ($p > 0.62$).

Cortical Analysis

ROI Analysis: Increased Thickness in Left Hemisphere Speech-Language Regions in GRIN2A

There was a significant overall group difference in cortical thickness in speech-language ROIs ($F_{4,54} = 5.85$, $p = 0.001$, Wilks' $\Lambda = 0.70$, partial $\eta^2 = 0.30$). Univariate analysis demonstrated increased cortical thickness in the pars opercularis ($F_{1,57} = 21.9$, $p < 0.0001$, partial $\eta^2 = 0.28$), supramarginal region ($F_{1,57} = 6.8$, $p = 0.01$, partial $\eta^2 = 0.11$), and superior temporal region ($F_{1,57} = 4.7$, $p = 0.03$, partial $\eta^2 = 0.08$) but not the precentral gyrus ($p = 0.12$).

There was a significant main effect of hemisphere ($F_{4,54} = 6.70$, $p < 0.001$, Wilks' $\Lambda = 0.67$, partial $\eta^2 = 0.33$) and a group by hemisphere interaction ($F_{4,54} = 0.2.9$, $p = 0.03$, Wilks' $\Lambda = 0.82$, partial $\eta^2 = 0.18$). Univariate analyses revealed that this interaction was significant only for the pars opercularis ($F_{1,57} = 8.88$, $p = 0.004$, partial $\eta^2 = 0.13$). Participants with pathogenic *GRIN2A* variants had thicker pars opercularis than controls, with a larger effect size in the left than in the right hemisphere (left: $F_{1,57} = 28.8$, $p < 0.0001$, partial $\eta^2 = 0.37$; mean (SD): *GRIN2A* = 2.97 (0.17), controls = 2.83 (0.13); right $F_{1,57} = 7.4$, $p = 0.008$, partial $\eta^2 = 0.12$; *GRIN2A* = 2.84 (0.18), controls = 2.83 (0.14)) (Figure 2). The secondary analysis with added IXI controls confirmed findings in the pars opercularis (eAppendix: Results A). There was no significant interaction effect of group by age ($p = 0.50$).

Whole-Brain Cortical Thickness Analysis

The group-level contrasts, before strict vertex-wise correction, revealed significant ($p < 0.0005$) clusters of increased thickness for participants with pathogenic *GRIN2A* variants in the left pars opercularis and orbitofrontal gyrus and bilateral increases in the superior frontal gyri and occipital gyri. Bilateral reductions were present in the superior parietal gyri (Figure 3A). After correction for multiple comparisons, only the bilateral increases in the lateral occipital gyri remained significant (left: cluster size = 1,467.5 mm^3, Talairach coordinates: x = −34.2 years = −79.9 z = 7.1; right: cluster size = 1,035.5 mm^3, Talairach coordinates: x = 27.4 years = −89.7 z = 1.9; $p = 0.01$) (Figure 3B).

ROI Analysis: Increased Pars Opercularis and Superior Temporal Volumes in Participants With *GRIN2A* Pathogenic Variants

Group differences in the *volumes* of speech-language regions were significant ($F_{4,53} = 3.47$, $p = 0.014$, Wilks' $\Lambda = 0.79$, partial $\eta^2 = 0.21$). The univariate analyses revealed larger volume in *GRIN2A* participants in the pars opercularis ($F_{1,56} = 9.44$, $p = 0.003$, partial $\eta^2 = 0.14$) and superior temporal region ($F_{1,56} = 4.51$, $p = 0.038$, partial $\eta^2 = 0.07$). There was no significant main effect of hemisphere ($p > 0.50$) and no interaction effects of hemisphere by age, sex, or group ($p > 0.12$). There was no significant interaction effect of group by age on cortical volume ($p = 0.25$). The secondary analysis with IXI controls confirmed pars opercularis differences only (eAppendix: Results B).

Whole-Brain Cortical Volume Analysis

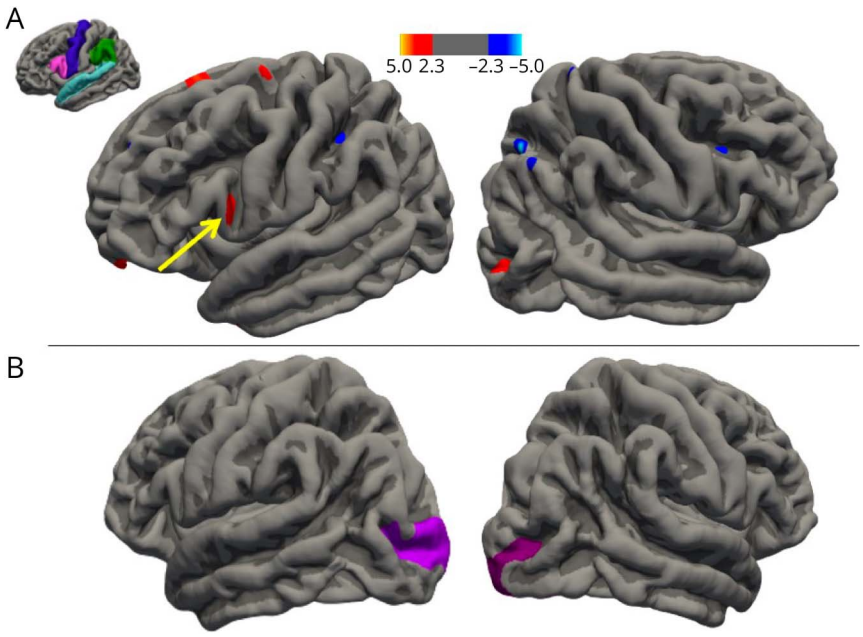
The group-level contrasts, without vertex-wise correction, revealed a significant cluster of increased cortical volume in the participants with pathogenic *GRIN2A* variants in the left superior temporal region ($p < 0.005$) and right temporal pole ($p < 0.0005$). After correction for multiple comparisons, no clusters remained significant.

Subcortical Analysis

Reduced Hippocampus in *GRIN2A* Participants

Multivariate ANCOVA revealed no overall effect of group ($p = 0.34$). Univariate analyses demonstrated decreased hippocampal volume in participants with pathogenic *GRIN2A* variants compared with controls ($F_{1,57} = 4.31$, $p = 0.04$, partial $\eta^2 = 0.07$; mean (SD) *GRIN2A* = 0.56 (0.02); controls = 0.60 (0.05)). *Post hoc* analysis including covariates revealed that the difference was driven only by the left hippocampus, which demonstrated a 20.6% reduction compared with controls

Figure 3 Group Differences in Cortical Thickness Using Whole-Brain Analysis: *GRIN2A* (n = 10) vs Controls (n = 51)



Left hemisphere shown on the left. (A) Thicker pars opercularis (arrow) in the *GRIN2A* group. Results are displayed before vertex-wise correction with significance set at $\log^{10}(2.3; p < 0.0005$; see color bar). Inset illustrates speech-language regions of interest. (B) Thicker bilateral occipital gyri in the *GRIN2A* group. Results are corrected for multiple comparisons with a Monte-Carlo simulation: vertex-wise significance level $p = 0.05$ and cluster-wise significance $p = 0.025$. Cluster significance: left, $p = 0.0006$; right, $p = 0.01$.

(left: $F_{1,57} = 5.99$, $p = 0.02$, partial $\eta^2 = 0.09$; mean (SD): *GRIN2A* = 0.272 (0.009), controls = 0.34 (0.026); right (ns $p = 0.11$)) (Figure 4). In the larger IXI control group, the results supported a left reduction (eAppendix C).

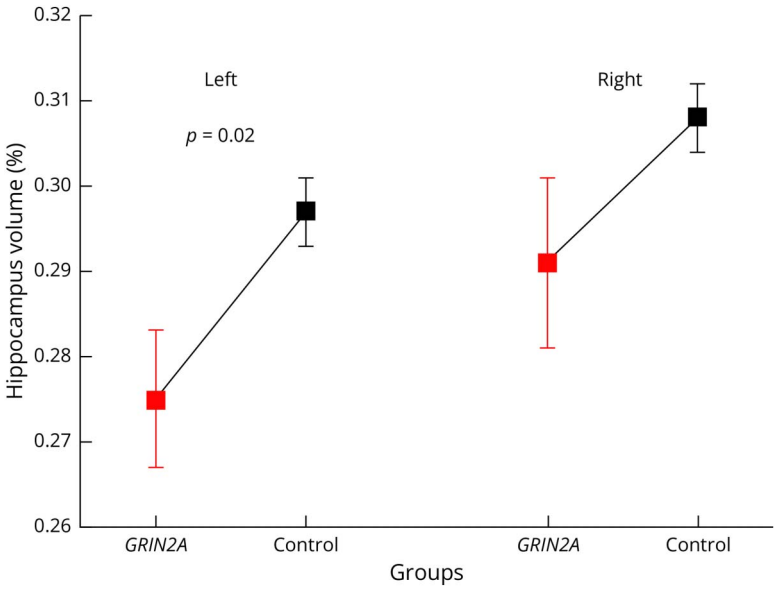
No Difference in Volume of the Corpus Callosum

There were no group differences in the volume of the corpus callosum ($p = 0.92$). This was confirmed in the secondary analysis with the larger control group ($p = 0.37$).

Discussion

Our study revealed that individuals with pathogenic *GRIN2A* variants and EAS have increased thickness and volumes in inferior frontal and superior temporal regions bilaterally, alongside volume reduction in the left hippocampus. The most robust and largest differences were found in the posterior part of Broca's area (pars opercularis) in the left hemisphere.

Figure 4 Estimated Marginal Means of Hippocampal Volumes in *GRIN2A* vs Controls (*GRIN2A* n = 10; Controls n = 51)



Y axis shows volume of left and right hippocampi as a percentage of total intracranial volume. Error bars depict standard error.

Alterations in the posterior part of Broca's area are consistent with the phenotype of CAS in our sample. The critical role of this region in speech production is well documented. Both functional MRI studies in healthy individuals and MRI analyses of adults with apraxia of speech post-stroke highlight the pars opercularis as key to speech planning and programming (see review[28]). A thicker superior temporal (planum temporale) cortex was also reported bilaterally in individuals with pathogenic *FOXP2* variants who also have persistent CAS, using voxel-based whole-brain methods.[22] The superior temporal and supramarginal regions, alongside the pars opercularis, are critical nodes within neuroanatomical models of speech production[29] and language processing.[32] These perisylvian regions form part of the dorsal language route, involved in sensory motor mapping of sound to articulation[33] and in the processing of complex grammatical structures.[34] Structural reductions and functional alterations along this dorsal route were recently reported in a family with inherited CAS without epilepsy.[17] In line with these findings, functional MRI underactivity in inferior frontal and supramarginal gyri is observed during speech tasks in adults with pathogenic *FOXP2* variants.[35] In the context of EAS, individuals with Rolandic epilepsy also show a thicker cortex in inferior frontal and supramarginal regions.[36] It should be noted, however, that MRI studies examining Rolandic epilepsy report inconsistent findings, with both increases and decreases[37] found depending on age (review by Smith et al.[38]). Altogether, the perisylvian anomalies reported here in individuals with pathogenic *GRIN2A* variants are consistent with their speech-language disorder and align with findings from other genetic conditions where phenotypes overlap. The neurodevelopmental process leading to increased cortical thickness in speech-related regions remains to be elucidated. Age-related thinning, especially within frontal regions, is well documented[39,40]; however, disruption to this process has been reported in various neurodevelopmental disorders such as autism-spectrum disorders [41] and schizophrenia.[42]

Suggested mechanisms which may inhibit cortical thinning in these populations could be either lack of pruning [43] or delayed brain development.[44] Unfortunately, because our sample was not longitudinal and included both adults and children, we are unable to reinforce evidence for either hypothesis. In our participants, cortical atypicalities could also be a cause or consequence of reduced functional network connectivity in the sensorimotor cortex, superior temporal gyrus, and pars opercularis, as demonstrated in children with Rolandic epilepsy during resting-state fMRI.[45] Although we are unable to pinpoint the mechanism of action causing increased cortical thickness in our population, we are confident that the absence of age-related effects indicates that perisylvian anomalies appear to be of a stable and persistent brain phenotype. This supports evidence of disrupted cortical thinning in families with speech disorders resulting from pathogenic variants.[46]

Clinical assessments of speech and language varied because of age and severity. A preliminary analysis between speech/language scores and cortical thickness of the perisylvian regions revealed a correlation between the ability to remember and produce nonwords (Nonword Memory Test[47]) and cortical thickness. However, the scores from this test were not standardized for age; as we saw both a correlation with age and cortical thickness and age and Nonword Memory Score, it would not be appropriate to infer the effect of cortical thickness on this language score. It is a limitation of this study that no evidence can be provided regarding the relationship between cortical morphometry and individual speech profiles for the individuals in this study.

In contrast to cortical enlargements, we detected volume reductions in the left hippocampus as predicted from animal models. In humans, this finding mirrors those reported in a child with a pathogenic *FOXP2* variant,[18] who showed bilateral reductions and additional reductions in the caudate nucleus and thalamus. Lesions of the left hippocampus in adults are not classically linked to aphasia, but rather to anterograde episodic memory impairment.[48] Similarly, early anomalies of the left hippocampus, such as those seen in children with temporal lobe epilepsy, are not classically associated with language impairments but rather verbal memory impairments (see review[49]). It should be noted, however, that an early role of the hippocampus in language learning has been suggested as part of the declarative/procedural model,[50] whereby declarative and procedural memory systems complement each other to support grammatical and lexical acquisition. For example, in healthy adults, the hippocampus is involved in the early stages of artificial grammar learning[51] and in language comprehension[52] because of its prediction and relational memory properties. In childhood, statistical learning abilities are linked to inferior frontal gyrus and right hippocampal measures.[53] There are also recent indications that the hippocampus is involved in speech feedback processing in adults.[54] We speculate that pathogenic *GRIN2A* variants alter the development of NMDA-dependent synaptic plasticity within the hippocampus,[55] which in turn hinders hippocampal development and its function in speech and language learning. Altogether, we provide further evidence that hippocampal reductions could act as biomarkers of genetic speech and language impairments.

The small sample analyzed in this study is a significant limitation. We tried to mitigate the risk of false positives using independent replication, but our study was underpowered to detect small effect sizes. While it may not be possible to generalize our findings to all variants of *GRIN2A* or other speech and language pathologies, our study combines deep phenotyping and advanced MRI in *GRIN2A*-related EAS to date. Alterations in the occipital cortex were not hypothesized and will require further investigation of visual processing in this patient population. In addition, larger samples would allow us to explore relationships between phenotype severity and brain metrics while longitudinal designs would allow us to detect whether brain anomalies precede EAS. Overall, pinpointing the mechanisms underlying the MRI anomalies

reported in this study could open new avenues for interventions targeting the dorsal language stream alongside hippocampal function.

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