Neuroanatomical anomalies associated with rare AP4E1 mutations in people who stutter

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Developmental stuttering is a common speech disorder with strong genetic underpinnings. Recently, stuttering has been associated with mutations in genes involved in lysosomal enzyme trafficking. However, how these mutations affect the brains of people who stutter remains largely unknown. In this study, we compared grey matter volume and white matter fractional anisotropy between a unique group of seven subjects who stutter and carry the same rare heterozygous AP4E1 coding mutations and seven unrelated controls without such variants. The carriers of the AP4E1 mutations are members of a large Cameroonian family in which the association between AP4E1 and persistent stuttering was previously identified. Compared to controls, mutation carriers showed reduced grey matter volume in the thalamus, visual areas and the posterior cingulate cortex. Moreover, reduced fractional anisotropy was observed in the corpus callosum, consistent with the results of previous neuroimaging studies of people who stutter with unknown genetic backgrounds. Analysis of gene expression data showed that these structural differences appeared at the locations in which expression of AP4E1 is relatively high. Moreover, the pattern of grey matter volume differences was significantly associated with AP4E1 expression across the left supratentorial regions. This spatial congruency further supports the connection between AP4E1 mutations and the observed structural differences.

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Abbreviations: AIBS = Allen Institute for Brain Sciences; DTI = Diffusion tensor imaging; FA = fractional anisotropy; GLM = General Linear Model; GMV = grey matter volume; VBM = Voxel-based morphometry

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Introduction

Developmental stuttering is one of the most common speech disorders, affecting about 5% of children and 1% of adults. It is a highly heritable disorder and likely to be a complex polygenic trait. Recently, persistent stuttering has been associated with mutations in the GNPTAB, GNPTG, NAGPA and AP4E1 genes. This set of genes is known to be involved in transporting lysosomal enzymes from the endoplasmic reticulum to lysosomes. Homozygous loss-of-function mutations in GNPTAB and GNPTG were previously known to cause the lysosomal storage diseases, Mucolipidosis Types II and III, while homozygous loss-of-function mutations in AP4E1 have been associated with spastic paraplegia and cerebral palsy. Patients with these mutations are characterized clinically by abnormal physical and cognitive development, including absent or delayed speech.

Rare genetic variations in the four genes associated with stuttering (GNPTAB, GNPTG, NAGPA and AP4E1) were found in ~20% of the unrelated cases of persistent stuttering whereas their incidences in the general population is <1%. Most of the variants identified in people who stutter are heterozygous missense mutations and not associated with any physical or cognitive abnormalities present in lysosomal storage diseases. On the other hand, accumulating neuroimaging evidence has shown that stuttering is associated with structural and functional anomalies in the brain regions involved in interhemispheric connections, language processing and speech–motor control. However, the connection between genetics and brain anomalies in people who stutter has not been established because genetic backgrounds of the participants in the previous neuroimaging studies were unknown.

In this case–control study, we used MRI to detect subtle neuroanatomical anomalies in a group of family members who all stutter due to the same genetic cause. They are all mutation carriers of the same two heterozygous mutations in AP4E1 gene (c.1549G>A and c.2401G>A) which, along with other rare mutations in this gene, have been shown to be associated with persistent stuttering in our previous genetic study. The control group was seven age-matched, unrelated normally fluent Cameroonians who do not have any of the AP4E1 variants or a history of stuttering. Two MRI techniques were used: (i)
high-resolution T1-weighted images for the measurement of grey matter volume (GMV) in cortical and subcortical areas and (ii) Diffusion tensor imaging (DTI) for the estimation of the fractional anisotropy (FA), which reflects microstructural coherence in white matter. Based on microarray gene expression data obtained from the Allen Institute for Brain Sciences (AIBS), we expected that the most prominent differences would be found in the thalamus and the corpus callosum where the expression of \( AP4E1 \) is the highest.\(^{19,20} \)

To further demonstrate that the differences are associated with the mutations instead of effects specific to the Cameroonian family, we quantified the spatial relationship between the pattern of GMV anomalies and \( AP4E1 \) expression levels across supratentorial brain regions. The gene expression data from the AIBS have been used as a proxy of genetic effects on different parts of the brain, revealing gene–brain relationships in several previous studies.\(^{17,18,21–24} \) For example, Grothe et al.\(^ {23} \) demonstrated that amyloid deposition in patients with Alzheimer’s disease is correlated with the expression of genes coding for the amyloid precursor protein. If mutated \( AP4E1 \) contributes to the neuroanatomical anomalies in the mutation carrier group, we would expect that the effects would be proportional to the levels of \( AP4E1 \) expression, leading to a spatial association between the pattern of GMV differences and \( AP4E1 \) expression in the brain.\(^ {17} \) Additionally, this relationship should be weaker in other gene associated with stuttering (\( GNPTAB, GNPTB \) and \( NAGPA \)).

**Methods**

**Standard protocol approvals, registrations and patient consents**

All participants were enrolled with written informed consent under National Institutes of Health (NIH) protocol 97-DC-0057 (ClinicalTrials.gov Identifier: NCT00001604) approved by the NIH Central Nervous System Institutional Review Board. Seven \( AP4E1 \) mutation carriers (five males and two females; mean age: 29.7 years; standard deviation: 7.7) with persistent neurodevelopmental stuttering from a large Cameroonian family were recruited.\(^ {3} \) All of them are heterozygous for the same \( AP4E1 \) mutation haplotype (c.1549G>A and c.2401G>A). Stuttering was diagnosed by speech pathologists using the Stuttering Severity Instrument Third Edition.\(^ {25} \) All the carriers displayed at least 4% dysfluency rate. Seven unrelated male non-carriers with no history of stuttering served as controls (mean age: 33.1 years; standard deviation: 10.1 years). They were recruited in the greater District of Columbia area in the USA and had immigrated to the USA from Cameroon within 6 months prior to their MRI measurements. Dideoxy sequencing was performed on \( AP4E1, \) \( GNPTAB, \) \( GNPTG \) and \( NAGPA \) to ensure that none of them carry a mutation in the known genes associated with stuttering. For both \( AP4E1 \) carriers who stutter and controls, their clinical history and physical examinations were conducted at the NIH Clinical Center, Bethesda, Maryland. Apart from persistent stuttering in the mutation carrier group, physical examinations were normal for all participants.

**Research procedures**

MRI images were acquired on a Siemens 3T MAGNETOM Skyra scanner with a 16-channel head coil at the NIH Clinical Center. Whole-brain T1-weighted images were collected using magnetization-prepared rapid gradient-echo sequence with the following parameters: Time of Echo (TE) = 1.76 ms, Time of Repetition (TR) = 5.1 ms, Flip Angle = 15°, Resolution = 0.98 × 0.98 × 1.0 mm. Seventy whole-brain diffusion-weighted images with \( b \) values of 300 or 1100 s/mm\(^2\) and 10 non-diffusion weighted volumes (\( b = 0 \)) were acquired in two runs using the following parameters: 80 axial slices, TR = 11.9 s, TE = 91 ms, Flip Angle = 90°, GRAPPA acceleration factor = 2, Resolution = 2 mm isotropic.

Voxel-based morphometry (VBM) analysis was performed using the CAT12 toolbox (http://www.neuro.uni-jena.de/cat/) and DARTEL normalization algorithm to obtain voxel-wise GMV.\(^ {26,27} \) Modulated GMV images were resampled to 1.5 mm isotropic voxels and spatially smoothed using a Gaussian kernel with a full-width half maximum (FWHM) of 6 mm. Voxels with mean grey matter probability less than 0.5 were excluded from further analysis. Group-level analysis of GMV was conducted using the General Linear Model (GLM) framework implemented by SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). Age, sex and intracranial volume were included in the GLM as nuisance variables. Since there were only two female participants in the mutation carrier group, including sex variable is important for capturing sex-specific effects. The initial voxel-wise thresholds were set at \( P < 0.005 \), and family-wise error (FWE) was corrected at the cluster level using Gaussian random field theory, corresponding to FWE-corrected \( P < 0.05 \).

Diffusion images were preprocessed using MRtrix \( dwi\)biascorrect script and FSL eddy commands.\(^ {28} \) MRtrix was used to estimate diffusion tensors and derive FA maps for each subject. The preprocessed FA maps were analysed using two complementary methods. First, we used FSL’s tract-based spatial statistics (TBSS) algorithm to project individual FA values greater than 0.25 to a pseudo-anatomical white matter skeleton.\(^ {29} \) FSL’s non-parametric permutation tool \( randomise \) was used to determine significant group differences.\(^ {30} \) Age and sex were included in the model as nuisance variables. Statistic threshold was set at FWE-corrected \( P < 0.05 \). In addition to tract-based analysis, we used a voxel-based analysis of FA to show the spatial extent of the group differences.
Individual FA images were normalized, resampled to $2 \times 2 \times 2$ mm resolution and spatially smoothed using a Gaussian kernel with a FWHM of 6 mm. Voxels with mean FA less than 0.25 were excluded from group analysis. SPM’s GLM with sex and age as nuisance variables was used for group analysis. Statistical significance threshold was set at FWE-corrected $P < 0.05$. We focussed on FA because most previous DTI studies on stuttering reported group differences in this measure.\(^{31}\) To complement the results of FA, we examined other DTI measures, including axial diffusivity (AD), radial diffusivity (RD) and mean diffusivity (MD).

**Association between AP4E1 expression and GMV differences**

Gene expression levels in different brain regions were obtained using the method previously described.\(^{17}\) Briefly, normalized microarray-based gene expression data from six adult donors (five males, one female; age: 24–57 years; see http://www.brain-map.org/ for details) were obtained from the AIBS.\(^{19,20}\) This data set contains expression of all protein coding genes in approximately 3700 samples from different brain regions. The anatomical locations of the samples were converted to the Montreal Neurological Institute (MNI) space by applying the transformation field obtained from DARTEL normalization of donor’s T1-weighted images using CAT12 toolbox. The expression of each gene from different probes was first averaged. The medians of all samples in each of the left cortical and subcortical regions defined by a standard atlas (AAL Atlas) were used to represent the overall expression level in the region.\(^{32}\) Regions in the right hemisphere were excluded because the majority of the samples were collected in the donors’ left hemispheres. The globus palladus was also excluded because it was consistently classified as white matter in all of our subjects. Similar to our previous study, regional GMV differences were obtained by averaging the magnitude of voxel-wise between-group $t$-statistics within each left supratentorial region defined by AAL Atlas.\(^{17}\) The association between the spatial patterns of AP4E1 expression and GMV differences across regions was evaluated using Spearman’s correlation.\(^{17}\) In addition, this correlation analysis was conducted on the other three genes associated with stuttering (GNPTAB, GNPTG and NAGPA) to show that the spatial relationship was specific to AP4E1.

**Data availability statement**

The datasets generated and/or analysed during the current study are available from the corresponding author upon request.

**Results**

We observed significant differences between AP4E1 mutation carriers who stutter and non-carrier, normally fluent controls in both GMV (Fig. 1A) and white matter diffusivity measures (Fig. 1B and C). The anatomical locations of these differences are listed in Tables 1 and 2. Compared to non-carrier controls, AP4E1 mutation carriers with persistent stuttering exhibited smaller GMV in the thalamus, the posterior cingulate gyrus and the calcarine gyrus (Fig. 1A). Furthermore, AP4E1 mutation carriers showed decreased FA in the corpus callosum in both tract-based (TBSS) and voxel-based analyses (Fig. 1B and C). Additionally, AP4E1 mutation carriers showed increased AD, RD and MD in the corpus callosum (Tables 1 and 2 and Supplementary Fig. 1). As expected, the regions that exhibited between-group differences in GMV and FA were the highly expressed locations of AP4E1 in the brain (Supplementary Table 1). In particular, the thalamus and the corpus callosum were the regions showing the highest expression of AP4E1 among the supratentorial regions defined by AAL atlas. Furthermore, the magnitude of between-group GMV differences and AP4E1 expression were moderately correlated ($r_s = 0.38, P = 0.011$) across supratentorial regions. This relationship is illustrated in Fig. 2. Since both AP4E1 expression and GMV difference were markedly larger in the thalamus than the other regions, we repeated the same analysis without the thalamus to ensure that the correlation was not solely driven by the thalamus. In this follow-up analysis, the spatial association was still significant ($r_s = 0.34, P = 0.028$), indicating that this relationship was not solely driven by the thalamus. Moreover, the spatial correlations between the patterns of GMV differences and expression patterns of the other three genes associated with stuttering were not significant (GNPTAB: $r_s = -0.15, P = 0.52$, GNPTG: $r_s = 0.21, P = 0.16$, NAGPA: $r_s = 0.14, P = 0.38$), and are illustrated in Supplementary Fig. 2.

**Discussion**

The objective of this study was to examine the neuroanatomical effects of specific heterogeneous AP4E1 mutations that were previously associated with persistent stuttering.\(^{3}\) We compared AP4E1 mutation carriers who stutter from a Cameroonian family with a group of unrelated, age- and ethnicity-matched, normally fluent, non-carriers who had no mutations in their AP4E1 gene. The ideal control group to compare with the carriers of AP4E1 mutations would be normally fluent members of the same family who do not carry the mutations. However, these individuals were not available for our study. While we are unable to rule out family-specific effects that are unrelated to the mutations by using unrelated Cameroonians as controls, we believe that the
observed neuroanatomical anomalies are likely to be associated with the AP4E1 mutation because the patterns of between-group structural differences appeared to be congruent with the expression of AP4E1 obtained from an independent sample. Specifically, not only were the largest between-group differences observed in brain regions where AP4E1 are highly expressed, i.e. the thalamus and the corpus collosum, but also the pattern of GMV differences across supratentorial regions was associated with the expression levels of AP4E1 in the brain. This congruency suggests that the structural differences between carriers and controls are potentially associated with these mutations in AP4E1 that have previously been associated with stuttering.

It is important to note that gene expression data used in the correlation analysis were not obtained from our participants because it is not feasible to take their brain tissue samples. Our observed spatial correlation between GMV differences and expression of AP4E1 does not imply a direct causal relationship between AP4E1 mutations and the AP4E1 expression levels in the carriers. Moreover, the gene expression data from AIBS are based only on six donors, and variability between donors may affect the representativeness of the data. However, up to now, the expression data from the AIBS represent the only comprehensive survey of gene expression in the human brain, and previous studies suggest that the patterns of gene expression in adult donors appear to exhibit a high degree of similarity.19,20,33
Are effects of \textit{AP4E1} mutations different from or the same as other causes of stuttering? Previous neuroimaging studies on people who stutter may give us some insight on this question. These previous studies did not genotype their participants, and thus their mutation status was unknown. However, the incidence of \textit{AP4E1} mutations in unrelated people who stutter is only 3.6% and we can assume that their contribution to the results of the previous neuroimaging studies is minimal. Thus, the previous results mostly reflect brain anomalies associated with mutations in genes other than \textit{AP4E1}, environmental effects or other factors. Several small studies have investigated GMV in adults who stutter with unknown mutation status. These studies reported that GMV differences between adults who stutter and controls were located in the frontotemporal areas or the caudate nuclei. In contrast, in our study, GMV differences were observed most prominently in the thalamus and the calcarine gyrus. This discrepancy indicates that effects of \textit{AP4E1} mutations on grey matter may be different from stuttering due to other causes and that different neural subtypes may exist in stuttering. This notion is further supported by a recent study in which the pattern of GMV differences between children who stutter (who were also not genotyped) and controls was shown to be significantly correlated with the expression of \textit{GNPTG} and \textit{NAGPA}, but not in \textit{AP4E1}. However, the results of the current study should be interpreted cautiously because of its small sample size. Moreover, there were only two female participants in the mutation carrier group, which limits our ability to examine potential interactions between sex and the effects of \textit{AP4E1} mutations. Larger imaging genetic studies are needed to confirm the unique and common effects of \textit{AP4E1} and their interactions with other factors.

Table 1. The locations and spatial extent of the significant differences between \textit{AP4E1} carriers and controls in the VBM and the voxel-based DTI analyses

| Region/MRI measure/contrast | Hemisphere | Peak x, y, z | Size (cm$^3$) | Corrected $P$ |
|-----------------------------|------------|--------------|---------------|--------------|
| Grey matter volume—Carriers $<$ Non-carriers | L/R | $-10, -30, 14$ | 58.7 | 0.001 |
| Thalamus | | | |
| Visual cortex and posterior cingulate cortex | L/R | $-2, -68, 14$ | 147.7 | $<$0.001 |
| FA—Carriers $<$ Non-carriers | L/R | $10, 24, -6$ | 20.3 | 0.002 |
| Genu and midbody of the corpus callosum | | | |
| Axial diffusivity—Carriers $>$ Non-carriers | L/R | $4, 4, 26$ | 43.2 | $<$0.001 |
| Genu, midbody and splenium of the corpus callosum | | | |
| Radial diffusivity—Carriers $>$ Non-carriers | L/R | $2, 2, 24$ | 77.1 | $<$0.001 |
| Genu and midbody of the corpus callosum | | | |
| Mean diffusivity—Carriers $>$ Non-carriers | L/R | $-2, -6, 26$ | 84.6 | $<$0.001 |
| Genu and midbody of the corpus callosum | | | |
| Splenium of the corpus callosum | L | $-24, -52, 10$ | 20.6 | 0.023 |

Table 2. The locations and spatial extent of the significant differences between \textit{AP4E1} carriers and controls in the tract-based analyses of DTI measures (TBSS)

| Region/MRI measure/contrast | Hemisphere | Peak x, y, z | # voxel (1 mm$^3$) | Corrected $P$ |
|-----------------------------|------------|--------------|-------------------|--------------|
| FA—Carriers $<$ Non-carriers | R | $21, 35, 12$ | 40 | 0.050 |
| Genu of the corpus callosum/anterior corona radiata | | | | |
| L | $-20, 36, 12$ | 13 | 0.050 |
| Axial diffusivity—Carriers $>$ Non-carriers | L/R | $13, 33, 8$ | 4041 | 0.002 |
| Genu and midbody of the corpus callosum | | | |
| Splenium of the corpus callosum | L/R | $-1, -38, 9$ | 64 | 0.044 |
| Radial diffusivity—Carriers $>$ Non-carriers | L | $-20, 35, 11$ | 1392 | 0.032 |
| Genu of the corpus callosum/anterior corona radiata | | | |
| R | $22, 33, 15$ | 341 | 0.040 |
| Mean diffusivity—Carriers $>$ Non-carriers | L/R | $-4, 24, 14$ | 7650 | 0.024 |
| Genu and midbody of the corpus callosum | | | |
| Splenium of the corpus callosum | R | $19, -36, 31$ | 322 | 0.042 |
Anatomically, this process could be achieved through projections from the premotor areas to the basal ganglia, which further project to the supplementary motor area (SMA) and pre-SMA via the thalamus. In support of this, direct electrical stimulation during awake brain surgery has demonstrated that transient stuttering-like dysfluencies can be elicited by stimulating a single region in the components of BGTC network, including the thalamus. These previous studies indicate that deficits in the thalamus may potentially contribute to the symptoms of stuttering.

Interestingly, compared to non-carrier controls, AP4E1 mutation carriers with persistent stuttering also exhibited smaller GMV in the calcarine gyrus and the posterior cingulate cortex. According to data from the AIBS, the expression of AP4E1 in these two regions is relatively high (see Supplementary Table 1), indicating that the GMV decreases are likely to be associated with the mutations. However, they are not typically associated with speech production and their involvement in stuttering is unclear.
In addition to GMV differences, reduced FA in the corpus callosum was observed in the carriers of AP4E1 mutations. It is consistent with a number of previous DTI studies of adults and children who stutter due to unknown causes.\textsuperscript{9,14,15,50–52} Moreover, a recent study showed that knock-in mice carrying Gnptab mutations, homologous to previously identified human stuttering mutations and functionally related to AP4E1, exhibited reduced astrocyte density and volume in the corpus callosum together with vocalization deficits similar to those in human stuttering.\textsuperscript{53} This animal study and our current study both indicate that structural abnormalities in the corpus callosum can be driven by specific genetic factors. However, the roles of the corpus callosum in speech production and stuttering are not fully understood. Perhaps, structural abnormalities in the corpus callosum could adversely affect hemispheric specialization of language,\textsuperscript{54} which have been hypothesized as a contributing factor in persistent stuttering.\textsuperscript{11}

In conclusion, this study provides preliminary evidence on the neuroanatomical effects associated with the AP4E1 mutations in people who stutter. Specifically, we showed that AP4E1 mutations were associated with anomalies in the brain structures previously linked to persistent stuttering, including the thalamus and the corpus callosum.

### Supplementary material

Supplementary material is available at Brain Communications online.

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### Competing interests

The authors report no competing interests.

### References

1. Frigerio-Domingues C, Drayna D. Genetic contributions to stuttering: The current evidence. Mol Genet Genomic Med. 2017;5(2):95–102.
2. Kang C, Riazuddin S, Mundorff J, et al. Mutations in the lysosomal enzyme–targeting pathway and persistent stuttering. N Engl J Med. 2010;362(8):677–685. doi:10.1056/NEJMoa0902630
3. Raza MH, Mattera R, Morell R, et al. Association between rare variants in AP4E1, a component of intracellular trafficking, and persistent stuttering. Am J Hum Genet. 2015;97(5):715–725.
4. Kornfeld S, Sly W. I-cell disease and pseudo-Hurler polydystrophy: disorders of lysosomal enzyme phosphorylation and localization. In: C Scriver, A Beaudet, W Sly, D Valle, eds. The Metabolic and molecular bases of inherited disease. New York: McGraw-Hill; 1995:2495–2508.
5. Reitman ML, Varki A, Kornfeld S. Fibroblasts from patients with I-cell disease and pseudo-Hurler polydystrophy are deficient in uridine 5'-diphosphate-N-acetylglucosamine: Glycoprotein N-acetylglucosaminylphosphotransferase activity. J Clin Invest. 1991;87(5):1574–1579.
6. Abou Jamra R, Philippe O, Raas-Rothschild A, et al. Adaptor protein complex 4 deficiency causes severe autosomal-recessive intellectual disability, progressive spastic paraplegia, shy character, and short stature. Am J Hum Genet. 2011;88(6):788–795.
7. Moreno-De-Luca A, Helmers SL, Mao H, et al. Adaptor protein complex-4 (AP-4) deficiency causes a novel autosomal recessive cerebro-palsy syndrome with microcephaly and intellectual disability. J Med Genet. 2011;48(2):141–144.
8. Raza MH, Domingues CEF, Webster R, et al. Mucolipidoses types II and III and non-syndromic stuttering are associated with different variants in the same genes. Eur J Hum Genet. 2016;24(4):529–534.
9. Chow HM, Chang S-E. White matter developmental trajectories associated with persistence and recovery of childhood stuttering. Hum Brain Mapp. 2017;38(7):3345–3359.
10. Garnett EO, Chow HM, Nieto-Castañón A, Tourville JA, Guenther FH, Chang SE. Anomalous morphology in left hemisphere motor and premotor cortex of children who stutter. Brain. 2018;141(9):2670–2684.
11. Neef NE, Anwander A, Bürfering C, et al. Structural connectivity of right frontal hyperactive areas scales with stuttering severity. Brain. 2018;141(1):191–204.
12. Kronfeld-Duenias V, Amir O, Ezraty-Vinacour R, Civier O, Ben-Shachar M. The frontal aslant tract underlies speech fluency in persistent developmental stuttering. Brain Struct Funct. 2016;221(1):365–381.
13. Beal DS, Gracco VL, Brtesschneider J, Kroll RM, De Nil LF. A voxel-based morphometry (VBM) analysis of regional grey and white matter volume abnormalities within the speech production network of children who stutter. Cortex. 2013;49(8):2151–2161.
14. Connally EL, Ward D, Howell P, Watkins KE. Disrupted white matter in language and motor tracts in developmental stuttering. Brain Lang. 2014;131:25–35.
15. Chang SE, Zhu DC, Chou AL, Angstadt M. White matter neuroanatomical differences in young children who stutter. Brain. 2015;138(Pt 3):e94–711.
16. Chang S-E, Angstadt M, Chow HM, et al. Anomalous network architecture of the resting brain in children who stutter. J Fluency Disord. 2018;55:46–67.
17. Chow HM, Garnett EO, Li H, et al. Linking lysosomal enzyme targeting genes and energy metabolism with altered gray matter
volume in children with persistent stuttering. *Neurol. Lang.* 2020;1(3):365–380.

18. Benito-Aragón C, Gonzalez-Sarmiento R, Liddell T, et al. Neurofilament-lysosomal genetic intersections in the cortical network of stuttering. *Prog. Neurobiol.* 2020;184:101718.

19. Hawrylycz MJ, Lein ES, Guillozet-Bongaarts AL, et al. An anatomically comprehensive atlas of the adult human brain transcriptome. *Nature.* 2012;489(7416):391–399.

20. Hawrylycz MJ, Miller JA, Menon V, et al. Canonical genetic signatures of the adult human brain. *Nat. Neurosci.* 2015;18(12):1832–1844.

21. Richardi J, Altmann A, Milazzo A-C, et al.; IMAGEN Consortium. *BRAIN NETWORKS.* Correlated gene expression supports synchronous activity in brain networks. *Science.* 2015;348(6240):1241–1244.

22. Ortiz-Terán L, Diez I, Ortiz T, et al. Brain circuit-gene expression relationships and neuroplasticity of multisensory cortices in blind children. *Proc. Natl Acad. Sci. U.S.A.* 2017;114(26):6830–6835.

23. Grothe MJ, Sepulcre J, Gonzalez-Ecamilla G, et al.; Alzheimer’s Disease Neuroimaging Initiative. Molecular properties underlying regional vulnerability to Alzheimer’s disease pathology. *Brain.* 2018;141(9):2755–2771.

24. McGolgan P, Gregory S, Seunarine KK, et al.; Track-On HD Investigators. Brain regions showing white matter loss in Huntington’s disease are enriched for synaptic and metabolic genes. *Biol. Psychiatry.* 2018;83(1):456–465.

25. Riley G. SSI-3: Stuttering severity instrument for children and adults, 3rd ed. Pro-Ed, Austin, TX; 1994.

26. Good CD, Johnsude IS, Ashburner J, Henson RN, Friston KJ, Frackowiak RS. A voxel-based morphometric study of ageing in 465 normal adult human brains. *Neuroimage.* 2001;14(1 Pt 1):21–36.

27. Ashburner J. A fast diffeomorphic image registration algorithm. *Neuroimage.* 2007;38(1):95–113.

28. Smith SM, Jenkinson M, Woolrich MW, et al. Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage.* 2004;23(Suppl 1):S208–19.

29. Smith SM, Jenkinson M, Woolrich MW, et al. Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data. *Neuroimage.* 2006;31(4):1487–1505.

30. Winkler AM, Ridgway GR, Webster MA, Smith SM, Nichols TE. Permutation inference for the general linear model. *Neuroimage.* 2014;92:381–397.

31. Neef NE, Anwander A, Friederici AD. The neurobiological grounding of persistent stuttering: From structure to function. *Curr. Neurol. Neurosci. Rep.* 2015;15(9):63.

32. Tzourio-Mazoyer N, Landeau B, Papathanassiou D, et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage.* 2002;15(1):273–289.

33. Kang HJ, Kawasawa YI, Cheng F, et al. Spatio-temporal transcription of the human brain. *Nature.* 2011;478(7370):483–489.

34. Lu C, Peng D, Chen C, et al. Altered effective connectivity and anomalous anatomy in the basal ganglia-thalamocortical circuit of stuttering speakers. *Cortex.* 2010;46(1):49–67.

35. Kell CA, Neumann K, Von Kriegstein K, et al. How the brain repairs stuttering. *Brain.* 2009;132(PT 10):2747–2760.