Amyotrophic lateral sclerosis (ALS) is a devastating, heterogeneous neurodegenerative neuromuscular disease that leads to a fatal outcome within 2–5 years, and yet, a precise nature of the association between its major phenotypes and the cerebellar role in ALS pathology remains unknown. Recently, repeat expansions in several genes in which variants appreciably contribute to cerebellar pathology, including C9orf72, NIPA1, ATXN2 and ATXN1, have been found to confer a significant risk for ALS. To better define this relationship, we performed MAGMA gene-based analysis and tissue enrichment analysis using genome-wide association study summary statistics based on a study of 27,205 people with ALS and 110,881 controls. Our preliminary results imply a striking cerebellar tissue specificity and further support increasing calls for re-evaluation of the cerebellar role in the ALS pathology.

Amyotrophic lateral sclerosis (ALS) is a devastating, heterogeneous neurodegenerative neuromuscular disease predominantly affecting upper and lower motor neurons, leading to death within 2–5 years. About 15% of people with ALS have mutations in one of the 40 Mendelian ALS genes. Recently, repeat expansions in several genes in which variants appreciably contribute to cerebellar pathology, including C9orf72, NIPA1, ATXN2 and ATXN1, have been found to confer a significant risk for ALS.

Cerebellar degeneration in ALS has long been a contentious topic, with the consensus being minimal involvement of the cerebellum in ALS, or at best, a compensatory role for cerebellar function during progressive supratentorial degeneration. This is, however, in opposition to compelling radiological and post-mortem pathologic evidence for extrapyramidal and cerebellar degeneration. Accordingly, a recent imaging study of 161 people with ALS, stratified for ALS-associated C9orf72 and ATXN2 variants, described distinct focal cerebellar trophic change, preferentially affecting specific lobules. Notably, a significant cerebellar pathology was also demonstrated in patients without these ALS-associated mutations.

Based on these findings, we explored whether significant cerebellar specificity of the ALS phenotypes could be confirmed by performing MAGMA tissue expression analyses on the ALS genome-wide association study (GWAS) summary statistics.

Results
The ten most statistically significant genes in MAGMA gene-based analysis were MOB3B, SCFD1, UNC13A, IFNK, G2E3, TNIP1, TBK1, BAG6 and EFTUD1. Complete list and MAGMA-dataset is available from https://fuma.ctglab.nl/browse/423.

Of 54 anatomical regions investigated, MAGMA-tissue-expression-profile-analysis revealed that the ALS-associated genes were significantly enriched for expression in the cerebellum and the cerebral-cortex [P(cerebellum) = 1.3 × 10^{-44}, P(cerebellar_hemispheres) = 1.5 × 10^{-44}, P(brain_frontal_cortex_BA9) = 3.3 × 10^{-44} and P(brain_cortex) = 1.2 × 10^{-44}]. This enrichment was observed even when known cerebellar pathology-associated ALS-risk genes C9orf72, ATXN1, ATXN2 and NIPA1 were excluded in later analyses to avoid disproportionate enrichment (Fig. 1). Notably, a significant cerebellar pathology was also demonstrated in patients without these ALS-associated mutations.

Based on these findings, we explored whether significant cerebellar specificity of the ALS phenotypes could be confirmed by performing MAGMA tissue expression analyses on the ALS genome-wide association study (GWAS) summary statistics.

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Discussion

We report a striking cerebellar tissue specificity for ALS. In addition, similar specificity is shown for the dorsolateral-prefrontal-region (the Broadmann-area-9), the cortical-area targeted with distinct cerebellar inputs via thalamic-projections, essential for 'higher'-cognitive functions such as working-memory, motor-planning, abstract reasoning and voluntary control of automatic movements.

Moreover, we report that this specificity remains even when we exclude ALS-genetic variants known to contribute to cerebellar pathology in ALS.

In past, ALS has been similarly associated with widespread and differential basal ganglia involvement. More specifically, changes in the regions of the nucleus caudatus, hippocampus, and in the region of the nucleus accumbens, have been proposed to present some of the key features of ALS. Accordingly, these brain regions feature among the top ten enriched anatomical regions (see Fig. 1, Table 1). Statistically significant specificity

![Figure 1. MAGMA tissue enrichment analysis of candidate genes for ALS, based on GTEx RNA-seq data of the 54 specific tissue types. Top 20 tissues are shown in figure. Significant tissues are marked with *.

| Anatomical region                        | Complete | No C9ORF72 | No C9ORF72,NIPA1,ATXN1,ATXN2 |
|------------------------------------------|----------|------------|-------------------------------|
| Brain cortex                             | 0.00012057 | 0.00011576 | 0.00011708                    |
| Brain cerebellum                         | 0.00013406 | 0.00017257 | 0.00017868                    |
| Brain cerebellar hemisphere              | 0.00015471 | 0.00019659 | 0.00020407                    |
| Brain frontal cortex BA9                 | 0.00033164 | 0.00033169 | 0.00033791                    |
| Brain nucleus accumbens basal ganglia    | 0.00098116 | 0.00092179 | 0.00092735                    |
| Brain anterior cingulate cortex BA24     | 0.0015641 | 0.0015002  | 0.0015101                     |
| Brain caudate basal ganglia              | 0.0023117 | 0.0021419  | 0.0021732                     |
| Brain putamen basal ganglia              | 0.0056439 | 0.0051024  | 0.005195                      |
| Brain hypothalamus                       | 0.0073602 | 0.0076545  | 0.007772                      |
| Brain hippocampus                        | 0.014025  | 0.01342    | 0.013692                      |
| Brain amygdala                           | 0.021646  | 0.020465   | 0.020641                      |
| Pituitary                                | 0.074125  | 0.079439   | 0.080076                      |
| Brain substantia nigra                  | 0.052635  | 0.12497    | 0.12759                       |
| Testis                                   | 0.16764   | 0.16718    | 0.16765                       |
| Cells EBV-transformed lymphocytes         | 0.18536   | 0.18119    | 0.18576                       |
has, however, only been demonstrated for the basal ganglia’s nucleus accumbens region (Table 1), and only in analyses that excluded the ALS-genetic variants known to contribute to cerebellar pathology in ALS, including C9orf72. This is perhaps somewhat contrainditive to previous studies, which argued a more intense basal ganglia involvement in patients with ALS carrying the C9orf72 hexanucleotide repeat expansion. Whilst the dissonance may reflect important aspects of our methodological limitations, we propose that our findings may also emphasise the complexity of the cerebellar role in the ALS-affected neurocircuity. For example, a specific (focal) cerebellar pathology may arguably dictate differential downstream changes in functional connectivity between the sub-regions of the cerebellum, the dorsolateral prefrontal cortex and the nucleus accumbens. The cerebellum shares functionality in motivated behaviors with these subcortical and cortical regions, and thus, any distinct cerebellar changes may drive and underlie, at least in part, different ALS phenotypes, with significant clinical implications.

In summary, the role of the cerebellum in exacerbating cardinal clinical manifestations such as motor disability, bulbar dysfunction, respiratory compromise, sleep and cognitive problems, is often overlooked, and symptoms traditionally primarily linked to supratentorial pathology. Furthermore, a closed-loop connectivity between localised regions of the prefrontal cortex, nucleus accumbens and cerebellum, and the extent to which cerebellar output may contribute to the ALS pathology remain mostly unmapped. Further aggravating point is that it is also challenging to identify cerebellar signs clinically in patients with motor weakness.

Our findings cannot be taken to suggest causality, or indeed the valence of these cerebellar associations due to the methodological limitations of MAGMA-analyses. Nonetheless, while cerebellar signatures of specific ALS-genotypes are yet to be firmly established, our study further supports increasing calls for re-evaluation of the cerebellar role in the ALS pathology.

Methods

For the purpose of this study, MAGMA gene-based analysis and tissue enrichment analysis were performed using genome-wide association study (GWAS) summary statistics from a study of 27,205 people with ALS and 110,881 controls, downloaded from https://surfdrive.surf.nl/files/index.php/s/E5RetKw10hC3Xye.

Three MAGMA-analyses were performed. During the first we analysed the entire GWAS-ALS dataset. To establish whether genes with known cerebellar involvement might be driving potential enrichment in cerebellum, we performed two additional analyses. For the first, all SNPs mapping positionally to C9orf72 were excluded (see Table 1, column ‘No C9orf72’. To the same end, additionally, all SNPs mapping positionally to C9orf72, ATXN1, ATXN2 and NIPA1 were excluded (see Table 1, the column ‘No C9orf72, ATXN1, ATXN2 and NIPA1’). MAGMA (v1.08) was invoked by FUMA (v1.3.7), an online tool for mapping and annotation of genetic associations. In MAGMA gene-based analysis, GWAS summary statistics are used to compute gene-based P values for protein coding genes by mapping SNPs to genes if SNPs are located within the genes. Bonferroni correction was used to correct for multiple testing.

Tissue-enrichment analysis was performed using the results of the gene-based analysis and the data from the Genotype—Tissue Expression (GTEx) project, integrated in FUMA (v1.3.7). GTEx project traditionally includes 54 specific human body tissue types, amongst which are thirteen different brain regions. Detailed information on the anatomical sampling sites, used databanks and the specific extraction methods can be found on https://www.gtexportal.org/. For example, for the cerebellar hemisphere please refer to https://www.gtexportal.org/home/tissue/Brain_Cerebellar_Hemisphere and for the cerebellum on https://www.gtexportal.org/home/tissue/Brain_Cerebellum.

Average gene-expression per tissue type was used as a gene covariate to test for a positive relationship between gene expression in a specific tissue type and genetic associations.

Ethics declarations. This study does not does report on experiments on humans. Only GWAS summary statistics have been used.

Data availability

The complete FUMA gene based and tissue based analysis results and parameters are available at https://fuma.ctglab.nl/browse/423.

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Author contributions
All authors conceived and planned the experiment. R.K. performed the analysis. All authors participated in writing the manuscript. All authors reviewed the manuscript.

Competing interests
The authors declare no competing interests.

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