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Genetic Associations With an Amyotrophic Lateral Sclerosis Reversal Phenotype

Jesse I. Crayle, MD,* Evadnie Rampersaud, PhD,* Jason R. Myers, MS, Joanne Wuu, ScM, J. Paul Taylor, MD, PhD, Gang Wu, PhD, Michael Benatar, MBChB, MS, DPhil, and Richard S. Bedlack, MD, PhD

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Correspondence
Dr. Crayle
jesse.crayle.md@gmail.com

Abstract

Background and Objectives

The term "ALS Reversal" describes patients who initially meet diagnostic criteria for amyotrophic lateral sclerosis (ALS) or had clinical features most consistent with progressive muscular atrophy (PMA) but subsequently demonstrated substantial and sustained clinical improvement. The objective of this genome-wide association study (GWAS) was to identify correlates of this unusual clinical phenotype.

Methods

Participants were recruited from a previously created database of individuals with the ALS Reversal phenotype. Whole-genome sequencing (WGS) data were compared with ethnicity-matched patients with typically progressive ALS enrolled through the CReATe Consortium's Phenotype-Genotype-Biomarker (PGB) study. These results were replicated using an independent ethnically matched WGS data set from Target ALS. Significant results were further explored with available databases of genetic regulatory markers and expression quantitative trait loci (eQTL) analysis.

Results

WGS from 22 participants with documented ALS Reversals was compared with the PGB primary cohort (n = 103) and the Target ALS validation cohort (n = 140). Two genetic loci met predefined criteria for statistical significance (two-sided permutation $p \leq 0.01$) and remained plausible after fine-mapping. The lead single nucleotide variant (SNV) from the first locus was rs4242007 (primary cohort GWAS OR = 12.0, 95% CI 4.1 to 34.6), which is in an *IGFBP7* intron and is in near-perfect linkage disequilibrium with a SNV in the *IGFBP7* promoter region. Both SNVs are associated with decreased frontal cortex *IGFBP7* expression in eQTL data sets. Notably, 3 Reversals, but none of the typically progressive individuals (n = 243), were homozygous for rs4242007. The importance of the second locus, located near *GRIP1*, is uncertain given the absence of an associated effect on nearby gene transcription.

Discussion

We found a significant association between the Reversal phenotype and an *IGFBP7* noncoding SNV that is associated with *IGFBP7* expression. This is biologically relevant as IGFBP7 is a reported inhibitor of the insulin growth factor-1 (IGF-1) receptor that activates the possibly neuroprotective IGF-1 signaling pathway. This finding is limited by small sample size but suggests that there may be merit in further exploration of IGF-1 pathway signaling as a therapeutic mechanism for ALS.

Trial Registration Information

This study was registered with ClinicalTrials.gov (NCT03464903) on March 14, 2018. The first participant was enrolled on June 22, 2018.

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*These authors contributed equally to this work.

From the Department of Neurology (J.I.C., R.S.B.), Duke University School of Medicine, Durham, NC; Department of Neurology (J.I.C.), Washington University in Saint Louis, MO; Center for Applied Bioinformatics (E.R., J.R.M., G.W.), St. Jude Children's Research Hospital, Memphis, TN; Department of Neurology (J.W., M.B.), University of Miami Miller School of Medicine, FL; and Department of Cell and Molecular Biology (J.P.T.), St. Jude Children's Research Hospital, Memphis, TN.

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Glossary

ALS = amyotrophic lateral sclerosis; **eQTL** = expression quantitative trait loci; **GWAS** = genome-wide association study; **LD** = linkage disequilibrium; **PGB** = Phenotype Genotype Biomarker study; **PMA** = progressive muscular atrophy; **SNV** = single nucleotide variant (formerly known as SNP); **WGS** = whole genome sequencing.

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by death of both upper and lower motor neurons, progressive weakness, and eventual respiratory failure. We previously reported a series of 36 patients[1] with a very rare ALS phenotype. These patients had a clinical course initially consistent with ALS or, in a minority of cases, progressive muscular atrophy (PMA; a lower motor neuron predominant variant of ALS[2]) and met established diagnostic criteria for ALS[3] or had diffuse lower motor neuron disease consistent with PMA.[4] Subsequently, these patients slowly attained partial or full clinical recovery. These improvements were both substantial and sustained,[1] and the observed disease course was not consistent with the natural course of ALS[5] or PMA.[4] Such rare ALS Reversal cases have been described since the 1960s.[6-18] To date, we have identified over 50 such cases with a unique ALS Reversal phenotype, and we have found that this phenotype does not have identifiable common environmental factors.[19] The biological basis for an ALS Reversal is unknown, but in the same way that genetic factors contribute to the etiology of ALS, we have speculated that genetic factors might similarly influence the probability of this unusual reversal of disease course. This study seeks to identify genetic correlates of the ALS Reversal phenotype by comparing whole-genome sequencing (WGS) data among a small cohort of ALS Reversals with 2 independent cohorts of patients with more typically progressive ALS. Such findings, although limited by the rarity of this phenotype, could guide future exploratory investigations of specific mechanisms of ALS disease resistance.

Methods

Participants

We previously constructed a database of patients with an ALS Reversal phenotype from published case reports and review of medical records.[1] Included patients were required to have initially met Awaji diagnostic criteria for ALS or had a clinical course consistent with PMA. Almost all patients had electrodiagnostic evaluation (i.e., nerve conduction studies and electromyography) performed, which was consistent with anterior horn cell disease and did not show evidence of conduction block suggestive of an immune-mediated motor neuropathy. The only patient who did not have an electrodiagnostic evaluation had upper motor neuron examination findings in cervical and lumbar regions. Neuroimaging was performed when appropriate to exclude multilevel radiculopathy with or without myelopathy. The reversal of ALS symptoms was defined by having met one of the following measures: a minimum of 4-point increase on the ALS Functional Rating Scale that was sustained over at least 6 months (see reference 5), a significant objective improvement in strength on manual muscle testing, an extraordinary improvement in gait (e.g., wheelchair bound improved to able to walk long distances) or other activities of daily living measures, or resolution of EMG denervation. Although patients were only required to improve on one measure, they could not worsen on other measures. Patients were excluded if their improvement was sudden (i.e., not consistent with gradual reinnervation), later worsening of disease was observed, or improvements were not sustained over follow-up. Of note, many patients plateaued in the recovery phase with some residual disability after significant and sustained improvement. All contactable patients with an ALS Reversal phenotype in our database were invited to participate in this genetic study named Study of ALS Reversals 2: Genetic Analyses (StAR2). Individuals who volunteered to participate were enrolled after obtaining informed consent.

Procedures

Demographics and disease characteristics were recorded by participant interview, and a detailed pedigree was taken if participants reported a family history of neurologic disease. Saliva samples were collected using the Oragene Saliva Collection Kit (DNA Genotek OGR-500), from which DNA was extracted. WGS was performed using the Illumina NovaSeq sequencing platform at the Hudson Alpha Genome Sequencing Center in collaboration with St. Jude's Children's Research Hospital. After WGS, we assessed for possible ALS disease-causing genetic variants. We also performed relationship inference using the KING-robust algorithm, with the KING software.[20]

Comparison Groups

For the planned analysis, the primary comparison group was a population of patients with ALS derived from the Clinical Research in ALS and Related Disorders for Therapeutic Development (CReATe) Consortium's Phenotype Genotype Biomarker (PGB) natural history study (NCT02327845). This study, which enrolled 527 patients with ALS (and additional patients with other related disorders), entailed collection of detailed longitudinal phenotypic data and generation of WGS data. To ensure appropriate comparisons, we restricted the comparative group to 103 patients with ALS in PGB for whom WGS was performed on the Illumina NovaSeq platform with adequate coverage and similar genome-based ethnicity estimates (i.e., European white ancestry—EUR) (eFigure 1, A and B). Ethnicity estimates were derived from the principal component analysis of common genetic markers measured in the

study samples against the 1000 genome reference data set.[21] To allow for further validation of potential significant findings resulting from the primary genetic analysis (Figure 1), we identified an independent ethnically matched cohort of 140 ALS non-Reversal patients, a subset from the Target ALS cohort. This data set was also sequenced using the Illumina NovaSeq platform.

Statistical Analysis

Standard GWAS-level QC was performed by limiting to common biallelic single nucleotide variant (SNV; formerly known as SNPs) (MAF ≥ 0.01 in the study cohort), excluding SNVs with high missingness (>5% missing data) and SNVs deviating from Hardy-Weinberg equilibrium ($p < 10^{-4}$). All analyses were performed using the plink v1.90b function "--assoc -perm" with adjustment for genomic sex, ALS age at onset, and PC1 and PC2 for genomic ethnicity.[22] Odds ratios were derived from this model. We used a standard genome-wide significance value of $p \leq 5 \times 10^{-8}$ and a permutation-based two-sided p-value threshold of ≤0.01 for the primary comparison with the PGB cohort (Figure 1). The secondary validation GWAS with the Target ALS cohort used a permutation-based two-sided p-value threshold of ≤0.01. Statistical analysis was performed using Microsoft Excel, JMP Pro Statistical Software (v15), and R Statistical Software (v4.1.0). The results were reported as per best practice Equator guidelines.[23]

Functional annotations of statistically associated SNVs were performed using expression quantitative trait loci (eQTL) results from brain cortex tissue in GTEx and MetaBrain data sets.[24,25] We also included ATAC-seq data from motor neurons derived from human-induced pluripotent stem cells (iPSC; GEO accession number GSE78036;[26]). Incorporating data from such databases allowed us to identify which noncoding variants from GWAS have potential regulatory roles for nearby genes.

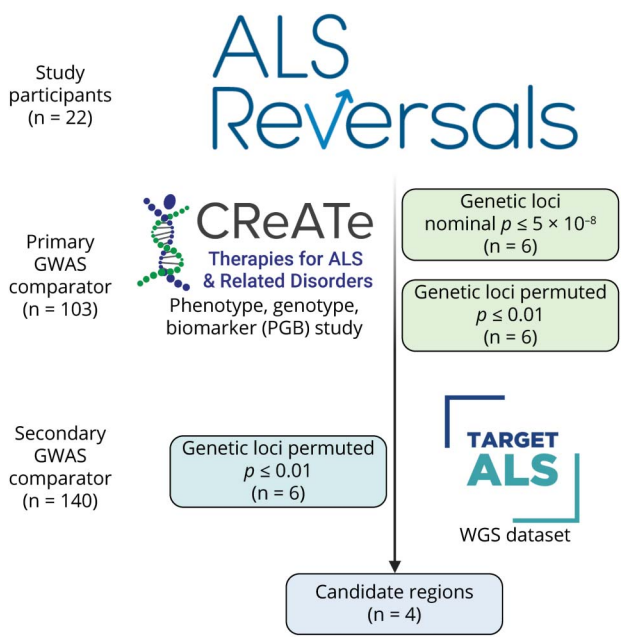
Standard Protocol Approvals, Registrations, and Patient Consents

This research study protocol was approved by the Duke University Health System institutional review board (Pro00091570). Informed consent was obtained from all participants in this study. This study is registered with ClinicalTrials.gov (NCT03464903).

Data Availability

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

Figure 1 Determination of Significant SNVs and Candidate Regions



Flowchart illustrates the analytic study design and filtering to the key genomic regions of interest. GWAS was conducted in 22 ALS Reversal patient compared with 103 ALS non-Reversal patients from the PGB cohort, resulting in 6 SNVs with nominal p-values passing genome-wide significance ($p \leq 5 \times 10^{-8}$). All 6 SNVs remained significant (PermP <0.01) after permutation-based association analysis testing. Six SNVs were also significantly associated (PermP ≤0.01; rounded to 0.01) when the ALS Reversal cases were compared with a separate ALS non-Reversal set of samples from the Target ALS cohort. These 6 SNVs fall in 4 regions of interest. Figure created with Biorender.com. ALS = amyotrophic lateral sclerosis; GWAS = genome-wide association study; PGB = Phenotype, Genotype and Biomarker study; SNV = single nucleotide variant.

Results

Participants

A total of 23 participants with previously documented ALS Reversal were successfully consented and enrolled. Twenty-two participants had WGS data with satisfactory technical quality (eFigure 1A). 73% of the participants in this genetic study (n = 16) were part of our prior descriptive study.[1] The remaining 6 participants in this genetic study were added to our ALS Reversal database after the publication of the prior study using the same criteria previously described[1] and summarized above. Of the 22 participants in this study, 4 had disease courses consistent with PMA[4] and the remaining (n = 18) met Awaji diagnostic criteria for ALS[3] before clinical improvement. Additional demographics and clinical characteristics of the 22 participants in this study were similar to the initially published cohort of 36 ALS Reversals (Table 1). As previously described, however, there was a higher proportion of White and male participants in the ALS Reversal cohort relative to cohorts of patients with more typically progressive ALS.[1] Among the 22 participants in this study, 7 had a known family history of neurologic diseases. Two participants (9%) had a family history of ALS (1 in a maternal cousin, 1 in a brother). Other participant-reported neurologic illnesses in these families included dementia (n = 3), Parkinson disease (n = 3), multiple sclerosis (n = 1), narcolepsy (n = 1), and essential tremor (n = 1). After WGS, we observed that 5 patients carried potential pathogenic or likely pathogenic variants in one of 140 genes associated with ALS or other neurodegenerative disorders with motor neuron involvement (eTable 1); however, only 1 patient was suspected to have a likely contributory variant after review of evidence from

existing literature, application of American College of Medical Genetics (ACMG) criteria,[27] and discussion with ALS experts in the CReATe Consortium genomics working group. This patient carried a rare coding variant (P392L) in *SQSTM1* (<0.2% in most populations in gnomAD[28]), which is possibly the most common *SQSTM1* sequence variant associated with ALS.[29] Outside of these 5 variants, there were no other pathogenic or likely pathogenic variants associated with diseases that might mimic ALS, such as spinal muscular atrophy, hereditary motor neuropathy, and hereditary spastic paraplegia. In our relatedness analysis, no first or second-degree relative pairs were identified, thus allowing for standard regression-based approaches to be used for association studies.

Genome-Wide Association Study

GWAS of the ALS Reversal participants and the primary comparison group (n = 103 PGB ALS non-Reversals) identified 6 SNVs meeting genome-wide significance (nominal $p \leq 5 \times 10^{-8}$) (Figure 1, Figure 2). All 6 SNVs were significant by permutation-based $p \leq 0.01$. In the secondary validation GWAS with n = 140 ALS non-Reversals from the Target ALS cohort, all 6 variants remained significant. These 6 significant SNVs were mapped to 4 different candidate regions. After fine-mapping and limiting to candidate regions with multiple significant SNVs in linkage disequilibrium (LD), 2 candidate regions remained (Table 2). One region was on chromosome 4 near *IGFBP7*, and the second region resided on chromosome 12 near *GRIP1/RAB11AP2* (Table 2). The candidate region near *GRIP1/RAB11AP2* was eliminated because of the SNV rs61918966 not having a demonstrable relationship with *GRIP1* mRNA expression in eQTL data sets of CNS tissue within either the GTEx (eTable 2, eFigure 2) or MetaBrain data sets (data not shown).

Table 1 Demographics and Clinical Characteristics of ALS Reversal Participants

		GWAS (n = 22)	Prior published series[1] (n = 36)
Sex, male	n (%)	16 (73%)	29 (81%)
Race, White	n (%)	21 (95%)	32 (88%)
Site of onset, limb	n (%)	20 (91%)	34 (94%)
Age at onset, y	Mean (SD)	51.0 (13.7)	50.1 (15.3)
ALS	n (%)	18 (82%) Criteria classification: *definite* n = 4 *probable* n = 12 *possible* n = 2	29 (81%) Criteria classification: *definite* n = 4 *probable* n = 23 *possible* n = 2
PMA	n (%)	4 (18%)	7 (19%)

Abbreviations: ALS = amyotrophic lateral sclerosis; GWAS = genome-wide association study; PMA = progressive muscular atrophy.
Of the 22 patients enrolled in this study, 16 were included in the prior published series.
Site of onset and diagnosis (before improvement) was determined based on previously abstracted data in our database of ALS Reversals. Sex, race, and age at onset for the 22 participants of this study are self-reported within this study.

The significant SNV in the remaining candidate region was rs4242007 (chr4:57107568, G/C), which is in intron 1 of *IGFBP7* (Figure 3). This SNV has a minor allele frequency of 8.1% among European (non-Finnish) individuals in the gnomAD database.[28] Of note, other persons with non-European ancestry seem to carry this minor allele at a much higher rate (approximately 35% in each African and East Asian populations). For the primary comparison, the odds of a person with the rs4242007 C allele showing reversal of ALS was 12.0 times greater than a person carrying the reference G allele (95% CI 4.1 to 34.6). Using the secondary validation cohort, the odds of a person with the rs4242007 C allele showing reversal of ALS was 3.3 times greater than a person carrying the G allele (95% CI 1.4 to 7.7). Of note, 3 ALS Reversal participants, but no individuals in the comparison PGB or Target ALS ethnically matched cohorts (combined n = 243), were homozygous for this SNV.

We also generated an additional GWAS (ALS Reversals vs PGB) with exclusion of the 4 PMA participants in the Reversal cohort to determine whether inclusion of these participants influenced the above finding because there were no PMA participants in either the PGB or Target ALS cohorts (eFigure 3). The lead SNV rs4242007 remained significant in this subanalysis ($p = 7.87 \times 10^{-9}$). On review of the individual-level data for rs4242007, 3 PMA reversal individuals were homozygous for the reference G allele and 1 was heterozygous for the alternate C allele. This suggests that including patients with a "PMA Reversal" did not meaningfully contribute to this study's finding.

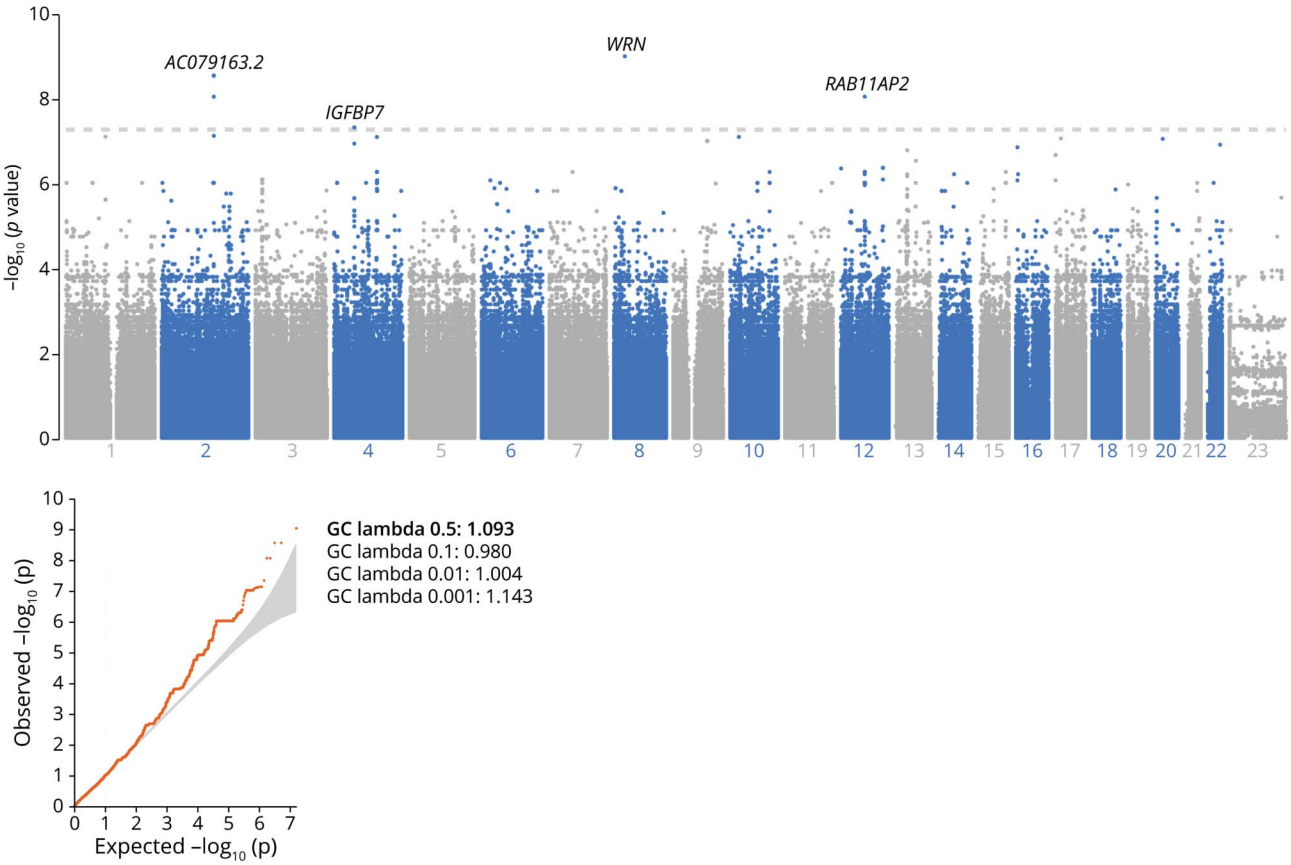
We then compared multiple self-reported variables including demographics, disease characteristics, comorbidities, and concomitant medications between enrolled ALS Reversals with and without the SNV. After adjusting for multiple comparisons, we found no significant differences in sex, race, site of onset, family history of ALS, or comorbidities (data not shown).

Secondary Analysis of GWAS Finding

In our study, the variant rs4242007 was in near-complete linkage disequilibrium ($r^2 = 0.98$) with rs4074555 (chr4:57110400, C/A), which itself approached significance in the primary GWAS ($p = 1.07 \times 10^{-7}$). Among the ALS Reversals, the 2 SNVs were in perfect LD. The lead SNV was also in moderate LD (0.40–0.80) with 7 additional associated SNVs ($p < 10^{-4}$) which further supports that this finding is not a false discovery. The SNV rs4074555 is part of the *IGFBP7* promoter region (located only 15 bp upstream from the gene) and is one of 4 common (MAF > 0.05) promoter region SNVs of *IGFBP7*.[30,31] In support of potential functionality, rs4074555 has a promoter-like signature in the ENCODE SCREEN Registry V3 when combining multiple data sources agnostic to cell type.[32] In addition, we reviewed a publicly available ATAC sequencing data set obtained from iPSC-derived motor neurons.[26] In this data set, rs4074555 appeared to be located within a *cis*-regulatory site (i.e., likely transcription factor binding site) adjacent to *IGFBP7* (Figure 3).

We, therefore, performed expression quantitative trait loci (eQTL) analyses with 2 data sets to assess for an effect of the

Figure 2 Primary GWAS Results



Manhattan plot depicts the results of GWAS comparing the CReATe PGB participants with ALS Reversals. Higher SNVs have smaller *p*-values, and the dashed horizontal line represents the genome-wide threshold of $p \leq 5 \times 10^{-8}$. The associated quantile-quantile plot demonstrates deviation of the observed vs expected *p* values, a measure of false positives. In this case, genomic inflation was within acceptable limits with the lambda genomic control statistic (50th percentile) less than 1.1. Figure created with Biorender.com. ALS = amyotrophic lateral sclerosis; GWAS = genome-wide association study; PGB = Phenotype, Genotype and Biomarker Study.

SNVs rs4242007 and rs4074555 on *IGFBP7* mRNA expression. In the GTEx data set,[24] there was a "dose-dependent" inverse relationship between the number of C alleles of rs4242007 and decreased expression of *IGFBP7* in brain cortex tissue ($p = 3.4 \times 10^{-5}$). Similarly, each additional A allele of rs4074555 was associated with decreased expression of *IGFBP7* ($p = 3.02 \times 10^{-7}$) (Figure 4). Multiple other brain tissues had a similar effect and nominal ($p < 0.05$) significance for an association between altered *IGFBP7* transcription and these 2 SNVs (eFigures 4 and 5, eTables 3 and 4). In the larger MetaBrain data set, which incorporates the GTEx data set,[25] the significant association between rs4242007 and *IGFBP7* expression in brain cortex tissue (European ancestry data set) was recapitulated ($p = 1.68 \times 10^{-27}$, $\beta = -0.53$). Similar results were found for rs4074555 ($p = 5.62 \times 10^{-23}$, $\beta = -0.52$), the promoter region SNV in near-perfect LD with rs4242007.

Discussion

In this study, we compared common genomic variants among patients with a unique ALS reversal phenotype against those from patients with ALS experiencing more typical disease progression. We found an enrichment of individuals carrying the C allele of rs4242007 among the ALS Reversal patients. We further showed that this intronic SNV is in almost perfect linkage disequilibrium with a promoter region SNV, rs4074555, and that both SNVs have significant associations with decreased expression of *IGFBP7* in eQTL studies, supporting their potential functionality as regulatory noncoding SNVs.

At the time of this study, we do not have neuropathologic data from patients with the ALS Reversal phenotype. In our opinion, one reasonable explanation for this clinical course is a pathologically less aggressive and clinically reversible form of ALS. Alternatively, the Reversal phenotype may represent a very rare reversible ALS mimic disease. The significant SNV in this study could be a positive risk factor for such a hypothetical disease.

IGFBP7 is a widely expressed coding gene with homology to insulin-like growth factor-binding proteins.[31] The gene protein product IGFBP7 likely functions as a noncompetitive inhibitor of the IGF-1 receptor with effects on downstream signaling.[33] Activation of this receptor leads to upregulation of

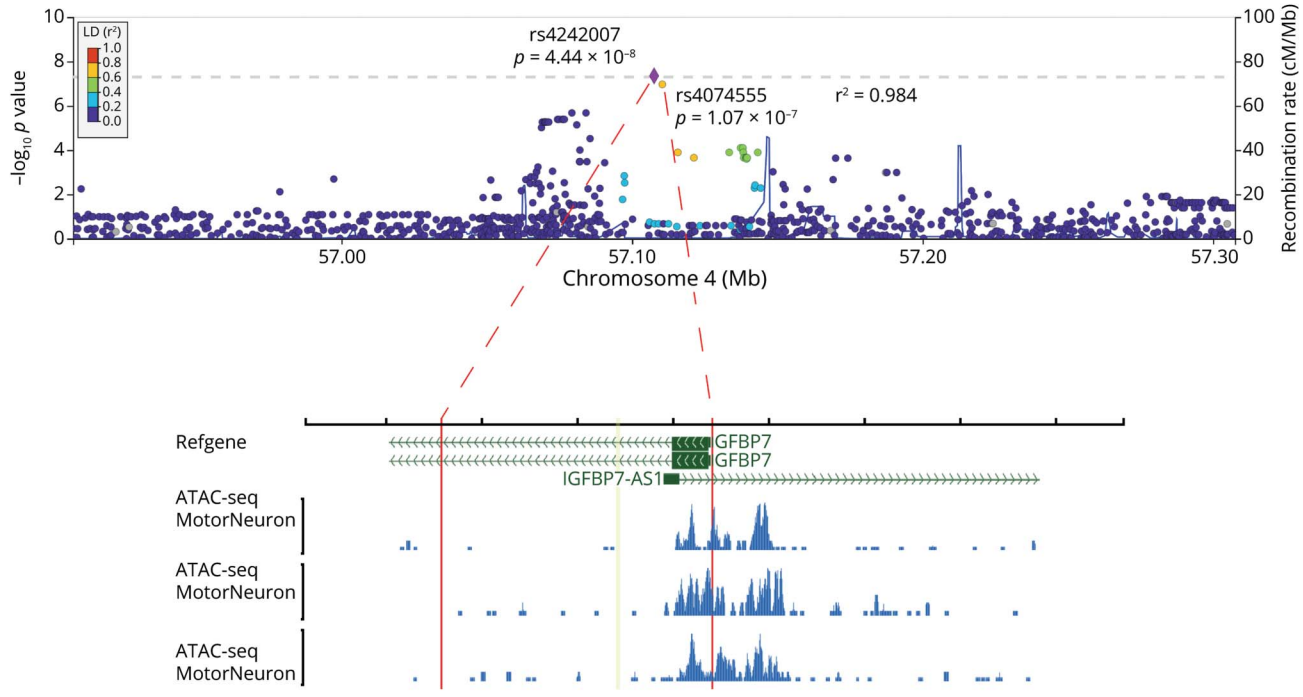
Table 2 Candidate Regions With Statistically Significant SNVs

Candidate Region	Outcome	CHR	BP	A1	A2	Comparison cohort 1: CReATe PGB study				Comparison cohort 2: Target ALS study			
						MAF(A)	MAF(U)	*p* Value	EMP	MAF(A)	MAF(U)	*p* Value	EMP
1	Excluded (single signal of adjacent SNVs)	2	145651891	C	T	0.167	0	2.67E-09	0.0085	0.167	0	6.13E-12	0.0019
			145651892	A	G	0.156	0	8.35E-09	0.0039	0.154	0	3.93E-11	0.0010
			145651895	C	A	0.167	0	2.67E-09	0.0085	0.167	0	6.13E-12	0.0019
2	**Candidate region near *IGFBP7***	**4**	**57107568**	**C**	**G**	**0.262**	**0.029**	**4.44E-08**	**1.50E-05**	**0.250**	**0.093**	**0.0047**	**0.0070**
3	Excluded (single associated SNV)	8	31100886	C	T	0.175	0	9.34E-10	1.00E-06	0.177	0	1.27E-12	1.00E-06
4	Candidate region without convincing eQTL data for altered brain expression of nearby genes	12	67149556	C	T	0.156	0	8.35E-09	0.0052	0.143	0.032	0.0055	0.0121

Abbreviations: A1 = Allele 1 from reference genome; A2 = Allele 2 which is the alternative allele; ALS = amyotrophic lateral sclerosis; BP = base pair; CHR = chromosome; EMP = empirical *p*-value, after permutation-based testing, which aims to reduce the false discovery rate; GWAS = genome-wide association study; MAF(A) = minor allele frequency, affected samples; MAF(U) = minor allele frequency, unaffected samples; PGB = Phenotype, Genotype and Biomarker; SNV = single nucleotide variant.
This Table 2 lists candidate regions determined by statistically significant SNVs in the GWAS between ALS Reversal participants and more typically progressive patients with ALS from the CReATe PGB study. These findings were validated by a second GWAS between the ALS Reversal participants and more typically progressive patients from the Target ALS database. Upon fine-mapping, candidate regions 2 and 4 were deemed plausible. We selected candidate region 2 (bold row) as the lead region of interest in this study. Candidate region 4 did not have significant cis-eQTL associations to allow for hypothesis generation.

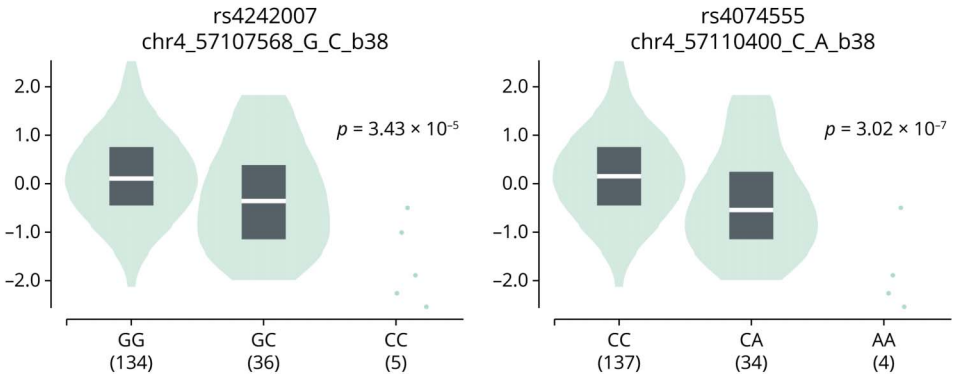
the PI3K-AKT and MAPK pathways, which regulate diverse cellular functions including prosurvival effects,[34] and are believed to play complex roles in ALS pathogenesis.[35,36] Although the role of IGFBP7 in ALS has not been fully elucidated, increased *IGFBP7* mRNA expression has been reported in 2 different mutant SOD1 mouse models of ALS

Figure 3 Fine-Mapping and ATAC-Sequencing



LD Zoomplot shows GWAS-associated SNVs within the region near *IGFBP7* on chromosome 4. The primary SNV rs4242007 is in almost perfect LD with rs4074555, a nearby upstream SNV, which overlaps peaks defining open chromatin regions from ATAC-seq experiments in motor neuron cell lines (iPSC-derived[26]). Figure created with Biorender.com. GWAS = genome-wide association study.

Figure 4 eQTL Analysis of Lead SNVs and IGFBP7 Expression in Frontal Cortex

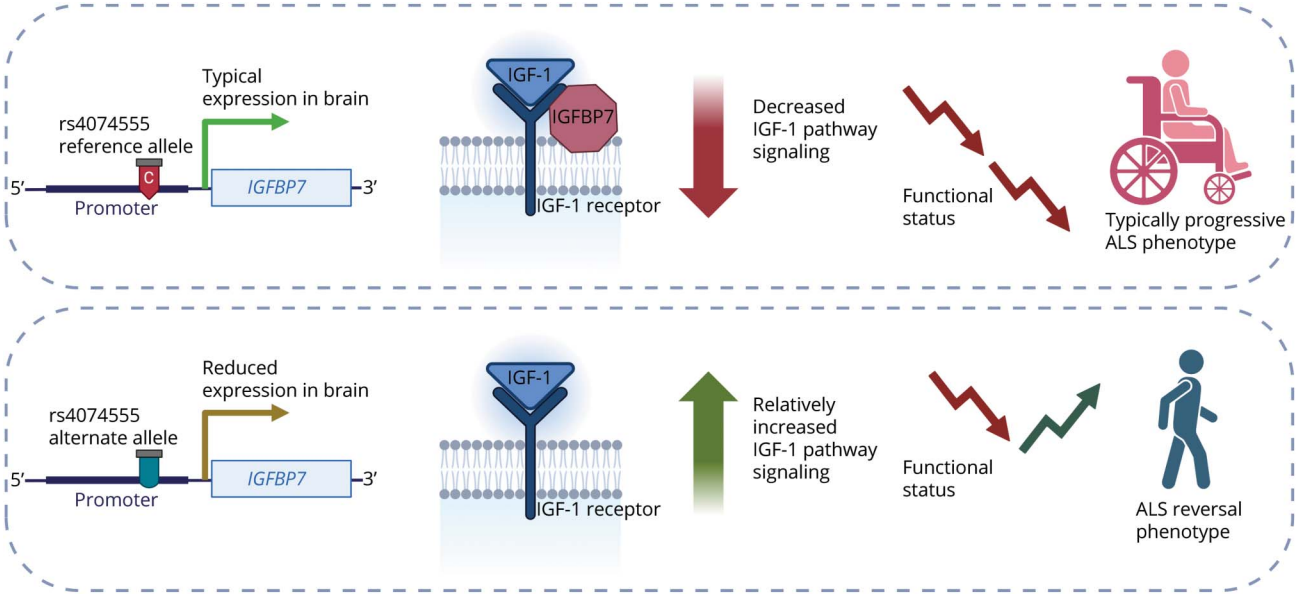


The GTEx database reports the C allele for single nucleotide variant (SNV) rs4242007 and the A allele of rs4074555 predict reduced expression of *IGFBP7* in brain frontal cortex (specifically Brodmann area 9). This appears to be an allele-dependent response. Other brain tissues appear to also have decreased expression in association with rs4242007, see eFigure 4. Figure created with Biorender.com.

(G37R and G85R).[37] In another neurodegenerative disease mouse model (Alzheimer disease double-mutant APP/PS1), hippocampal IGFBP7 protein levels were reportedly elevated. Intriguingly, anti-IGFBP7 antibody injected into the hippocampus of this Alzheimer mouse model was reported to improve memory task performance while injection of the IGFBP7 protein worsened performance.[38]

Given the abovementioned suggestions of a deleterious effect of IGFBP7 in neurodegenerative disease coupled with IGFBP7's putative inhibition of IGF-1 signaling,[33] we hypothesize that a reduction in IGFBP7 expression with a resulting increase in IGF-1 signaling might be beneficial in slowing the rate of ALS progression. Although the neuropathology of ALS Reversals is not known, our study's findings suggest that this IGF-1 signaling pathway may also contribute to the phenotype of ALS Reversals in a subset of cases (Figure 5). Decreasing IGFBP7 has not been specifically tested in ALS models; there are preclinical data showing that increasing IGF-1 in the CNS has benefit for the mutant SOD1 mouse model of ALS.[39-41] In addition, in one cohort of people with ALS (PALS), higher serum IGF-1 levels seem to be associated with longer survival.[42] There have been 4 clinical trials in PALS to assess for a clinical effect of IGF-1 on ALS disease progression. The first 2 trials were reasonably well conducted using 0.05–0.1 mg/kg/d of subcutaneous IGF-1 but reported mixed results on Appel ALS rating scale scores,[43,44] with a possible benefit at the higher dose.[45] A subsequent well-conducted trial using a motor scale failed to recapitulate this

Figure 5 Hypothesized Relationship of Allele-Specific rs4074555 Effects on IGFBP7 and IGF-1 Expression



Schematic of hypothesized role for the promoter region SNV rs4074555 alternative allele in reducing expression of *IGFBP7* leading to the subsequent relative increase of IGF-1 signaling, which we hypothesize provides a benefit in patients with ALS. As described in this study, the intronic SNV rs4242007, in near-perfect LD with rs4074555, has the same effect in eQTL analyses. Figure created with Biorender.com. ALS = amyotrophic lateral sclerosis; SNV = single nucleotide variant.

benefit.[46] However, some investigators have pointed out that these doses are unlikely to have biologically relevant CNS penetration, and IGF-1 has a very short serum half-life.[47] A small (n = 9) double-blind randomized two-arm drug trial (high vs low-dose) in Japan reported a small benefit on Norris ALS scale scores with intrathecal IGF-1,[48] but this has not been reproduced and the small size of this trial without placebo comparison precludes any firm conclusions.

While ALS Reversals have been described in the literature since the 1960s, to our knowledge, this is the first report of a genomic analysis in this ultra-rare population. In our study, ALS Reversals were significantly more likely to have a specific SNV in the first intron of *IGFBP7* than patients in 2 independent cohorts with more typical ALS. Of note, as in other observational prospective cohorts of ALS, we have noted that PGB is slightly enriched for more slowly progressing patients.

This study was chiefly limited by the obligatorily small size of the ALS Reversal group. This resulted in being underpowered for rarer SNV associations. Another limitation is that the cohort of ALS Reversals predominantly comprises White and male participants. Although we ensured ancestry matching with patients with ALS who have more typical disease courses, it remains unknown whether this SNV would be associated with the phenotype of ALS Reversals in a more diverse cohort because the minor allele frequency is much higher in African and East Asian populations. The demographics of the ALS Reversal cohort do not exclude the possibility of ALS Reversal occurring in non-White patients with ALS. Owing to necessary reliance on literature reports, provider referrals and self-report for identification of potential ALS Reversal, there may be under-discovery of non-White and female ALS Reversals because of socioeconomical inequalities in medical care access and delivery. Despite these limitations, this exploratory study is strengthened by a rigorous statistical process that exceeds GWAS best practice by replicating the results against a validation cohort.

In summary, we found an *IGFBP7* intronic SNV that is significantly associated with the phenotype of ALS Reversal. This SNV is in near-perfect LD with an *IGFBP7* promoter region SNV which itself approached statistical significance in this study. Furthermore, carrying the alternative allele of either SNV is associated with decreased expression of *IGFBP7* in the frontal cortex in eQTL data sets. We propose that allelic additivity for this *IGFBP7* regulatory noncoding SNV rs4242007 may, in a subset of ALS Reversal, partially explain this unique phenotype through facilitation of IGF-1 signaling. The relevance of this finding to patients with typically progressive ALS is unknown; prior IGF-1 trials in PALS have shown mixed results but may not have been optimally dosed. In the future, we plan to further define the biological nature of ALS Reversal and explore the hypothetical mechanism of IGF-1 signaling with the hope that this could be used in the future to underpin an ALS therapeutic strategy.

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Appendix Authors

Name	Location	Contribution
Jesse I. Crayle, MD	Department of Neurology, Duke University School of Medicine, Durham, NC; Department of Neurology, Washington University in Saint Louis, MO	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data
Evadnie Rampersaud, PhD	Center for Applied Bioinformatics, St. Jude Children's Research Hospital, Memphis, TN	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data
Jason R. Myers, MS	Center for Applied Bioinformatics, St. Jude Children's Research Hospital, Memphis, TN	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data
Joanne Wuu, ScM	Department of Neurology, University of Miami Miller School of Medicine, FL	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data
J. Paul Taylor, MD, PhD	Department of Cell and Molecular Biology, St. Jude Children's Research Hospital, Memphis, TN	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data
Gang Wu, PhD	Center for Applied Bioinformatics, St. Jude Children's Research Hospital, Memphis, TN	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data
Michael Benatar, MBChB, MS, DPhil	Department of Neurology, University of Miami Miller School of Medicine, FL	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data
Richard S. Bedlack, MD, PhD	Department of Neurology, Duke University School of Medicine, Durham, NC	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data

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